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Asymptomatic shedding of herpes simplex virus (HSV) in the oral cavity

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Objective. The aim of this study was to investigate the rate of herpes simplex virus (HSV) shedding from the oral cavity, because recent studies suggest that shedding is more frequent than originally reported. Factors that could influence the rate and duration of shedding from the oral cavity were examined.
Methods. Existing epidemiologic data from 22 reports of HSV shedding from more than 3,500 individuals were analyzed with regard to demographics, frequency of sampling, and methodologic assays.
Results. HSV-1 was more likely to be detected than HSV-2 in the oral cavity of asymptomatic persons (7.5 odds ratio, 95% confidence interval 4.4-12.8; $P < .0001$). The rate of shedding was highly variable among individuals, ranging from none to 92% of days tested, and occurred in seropositive and seronegative individuals. In cell culture studies, the rate of detection on a single day was 6.3%. Polymerase chain reaction studies provided a different picture. HSV-1 DNA was present in 97 of 180 patients (53.9%) at multiple visits, with a rate of daily detection of 33.3%. The mean duration of shedding was between 1 and 3 days, but more than 3 days in about 10% of patients.
Conclusions. At least 70% of the population shed HSV-1 asymptomatically at least once a month, and many individuals appear to shed HSV-1 more than 6 times per month. Shedding of HSV-1 is present at many intraoral sites, for brief periods, at copy numbers sufficient to be transmitted, and even in seronegative individuals. The dental implications of these findings are discussed. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:43-50)**

Herpes simplex virus (HSV) is a large enveloped DNA virus and significant human pathogen. It infects most persons early in life, primarily at mucosal surfaces following exposure to infected secretions, and causes a range of diseases from labialis and stomatitis to blinding keratitis and, rarely, encephalitis. Over 70% of the adult population have neutralizing antibodies to HSV and serve as reservoirs of the virus.[1-4] Despite several host immunological factors (i.e., neutralizing antibodies and T_H2 cell–mediated immunologic responses), HSV persists for the life of its host.[5] Reasons for its success include: 1) HSV is a pathogen that replicates preferentially in epithelium but can migrate and replicate in other cell types; 2) HSV seldom kills its human hosts; and 3) the virus can hide indefinitely in several neuronal sites and possibly other tissues of the host.[6] Key to its perpetuation is its ability to episodically reactivate from latency. Virus reactivation results in the asymptomatic shedding and/or the development of recurrent orofacial herpes infections. About 25% of seropositive persons develop recurrent infections 1 to 4 times per year,[1,2,7] and typically these occur after stressful events, trauma, febrile illness, or immunesuppression.[2,8,9] In contrast, many individuals do not report recurrent lesions, suggesting that reactivation either does not occur or that the resulting progeny fail to cause recurrent lesions in select individuals.

Asymptomatic shedding is generally defined as the presence of HSV in the absence of clinical lesions.[10,11] Sites of shedding include the mucosal surfaces of the eyes,[12] mouth,[13] and genitalia.[14] The frequency of shedding is site specific and reflects the frequency of virus

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reactivation and the level of immune control.[5,11] The rate of asymptomatic HSV shedding from the oral cavity is more frequent than the rate of clinical orofacial recurrences (i.e., lesions).

In an earlier review,[15] asymptomatic HSV shedding in the oral cavity was reported to occur in approximately 5% of individuals. This low prevalence figure is based largely on studies that sampled patients infrequently and used cell culture techniques as the primary method for virus detection. Over the last decade, detection methods have improved and sampling frequencies have increased, resulting in a greater understanding of the rate of HSV shedding in the oral cavity. In the present review, we analyzed studies that examined rates of HSV-1 shedding and factors that influenced the rate of viral shedding in the oral cavity. We found that asymptomatic HSV-1 shedding is more frequent than previously believed and is regulated by specific factors. The implications of these findings with respect to HSV reactivation, transmission, and the practice of dentistry are briefly discussed.

METHODS

Identification and selection of studies

The medical literature from 1953 through December 31, 2006, was searched, using PubMed, for publications in the English language that reported on HSV-1 presence in the oral cavity or mouth. In PubMed, the keywords herpes simplex virus, oral, mouth, shedding, saliva, and recurrence were searched alone and in combination. Reference lists of relevant publications and review articles were examined to identify additional studies. Twenty-four articles published in peer-reviewed journals between 1953 and December 2006 were identified. These were evaluated critically by one of the authors (C.S.M.). Studies were included for analysis if culture or molecular analyses were used to detect HSV in cells derived from saliva or oral mucosa. Studies were excluded if: 1) patients had a known disease (i.e., human immunodeficiency virus (HIV) infection, immunosuppression) that could alter the frequency of asymptomatic shedding; 2) the study cohort included men who had sex with men (MSM); 3) shedding was associated with documented recurrent lesions (e.g., vesicles or ulcers); or 4) the publication was not in English. Data were also excluded if samples were derived from nonoral sites (i.e., tonsillar, pharyngeal, or aerodigestive tissue) or if insufficient information was available to ascertain a definitive result. The following data were recorded: authors, patient age, sample size, country, sampling frequency, prevalence at single or multiple visits, duration of sampling, method of detection, and viral genome copy numbers. In this report children are defined as aged >6 months and ≤14 years and adults are defined as aged >14 years.

Statistical analyses

Odds ratios were calculated to determine if differences in the ability to detect HSV corresponded with single versus multiple visits or cell culture versus nucleic acid amplification using the Cochran-Mantel-Haentszel statistic. Subsequently, the Breslow-Day statistic was computed to test the hypothesis that the odds ratio was the same or different across the strata. A chi-squared test was performed to determine if sampling more than 5 times per week was more sensitive for the detection of HSV than sampling fewer than 5 times per week.

RESULTS

Characteristics of the studies

This study analyzed the rate of asymptomatic shedding of HSV in the oral cavity in otherwise healthy individuals. Excluded were studies that evaluated individuals positive for HIV and/or MSM.[16,17] Select characteristics of the 22 studies that met the criteria for analysis are shown in Tables I and II. In total, 14,404 samples derived from saliva or swabs of the oral mucosa were analyzed. All studies were analyzed independently. Age was identified in less than 20% of the study populations. In many studies, only the mean age of the study cohort was provided and was suggestive that the sample was primarily from adults. Although most studies included both men and women, insufficient data was provided for analysis of gender differences. The method of detection was reported in all studies, with culture techniques used in 10 studies and nucleic acid amplification used in 13 studies, primarily in the more recent studies. One study employed both assay methods. Ten studies reported sampling asymptomatic patients at 1 visit, with 1,861 patients analyzed for HSV-1 and 744 patients analyzed for HSV-2. Thirteen studies reported sampling multiple times, with 1,708 patients analyzed for HSV-1 and 247 patients analyzed for HSV-2. Of note, the studies that performed multiple-day sampling often included patients who developed HSV recurrences during the study. To appropriately determine asymptomatic shedding rates in these individuals, data associated with the development of oral HSV recurrent lesions were excluded from the analyses.

Frequency and type of HSV shedding in the oral cavity at a single time point

Based on the reported detection rates of HSV-1 and HSV-2, HSV-1 was more commonly detected in the

Table I. Asymptomatic shedding of HSV-1 from the oral cavity of otherwise healthy individuals

Investigator	Age	N	Country	Individuals (%) positive for HSV as reported by frequency and duration: 1 visit	Individuals (%) positive for HSV as reported by frequency and duration: ≥3 visits	Method of detection
Buddingh et al. 1953[13]	C	368	USA		34 (9.2%) weekly or twice weekly intervals for several months	Culture
Buddingh et al. 1953[13]	A	185	USA		5 (2.4%) weekly or twice weekly intervals/for several months	Culture
Kaufman et al. 1967[12]	NR	35	USA		6 (17.1%) weekdays (5) for 20 days (3 weeks)	Culture
Lindgren et al. 1968[39]	A	418	USA		8 (1.9%) daily and every other day for 3 to 5 weeks	Culture
Douglas and Couch 1970[40]	A	10	USA		8 (80%) 3X week for 5 months	Culture
Hatherley et al. 1980[20]	A	384	Australia		37 (9.6%) weekly for 5 weeks	Culture
Spruance 1984[8]	A	8	USA		8 (100%) weekdays for 5 months	Culture
Kameyama et al. 1988[21]	A	110	Japan		5 (4.5%) daily for 2 months	Culture
Robinson et al. 1992[41]	NR	12	United Kingdom	0 (0%)		PCR
Tateishi et al. 1994[24]	A & C	1000	Japan	46 (4.6%)/27 (2.7%)		PCR/culture
Kriesel et al. 1994[9]	A	27	USA	3 (11.1%)		PCR
Lee et al. 1996[42]	NR	87	Korea	12 (13.8%)		PCR
Okinaga 2000[22]	A	10	Japan		4 (40%) 3X weekly for up to 6 months	Culture
Knaup et al. 2000[43]	A	30	Germany		13 (43%) about every 10 days for 2-6 months	PCR
Gleeson et al. 2002[44]	A	14	Australia		0 (0%) every 2 to 3 days for 30 days	PCR
Youssef et al. 2002[45]	A	5	Egypt	1 (20%)		PCR
Druce et al. 2002[33]	NR	477*	Australia	43 (9.0%)		PCR
Miller et al. 2004[23]	A	123	USA	11 (8.9%)		PCR
	A	61			13 (21%) every 3 to 4 days for 1 week	PCR
Kaufman et al. 2005[19]	A	50	USA		46 (92%) twice daily for 30 days	PCR
Da Silva et al. 2005[28]	A	25			25 (100%) every 30 to 60 days for 8 to 11.5 months	PCR
Lin et al. 2005[46]	NR	72	Taiwan	0 (0%)		PCR
Miller et al. 2005[26]	A	58	USA	1 (1.7%)		PCR
Total				117/1861 (6.3%)	212/1708 (12.4%)	

Data from HSV seropositive and seronegative patients.
A, Adults (>14 years old); *C*, children (>6 months to 14 years old); *NR*, not reported.
*Health status of patients not identified.

Table II. Asymptomatic shedding of HSV-2 from the oral cavity of otherwise healthy individuals

Investigator	Age	N	Country	Individuals (%) positive for HSV as reported by frequency and duration: 1 visit	Individuals (%) positive for HSV as reported by frequency and duration: ≥3 visits	Method of detection
Docherty et al. 1985[29]	A	12	USA	0 (0%)		Culture
Kameyama et al. 1988[21]	A	110	Japan		0 (0%) daily for 2 months	Culture
Gleeson et al. 2001[44]	A	14	Australia		0 (0%) every 2 to 3 days for 30 days	PCR
Druce et al. 2002[33]	NR	477*	Australia	3 (0.6%)		PCR
Miller et al. 2004[23]	A	123	USA	0 (0%)	0 (0%) every 3 to 4 days for 1 week	PCR
Lin et al. 2005[46]	NR	132	Taiwan	0 (0%)		PCR
Total				3/744 (0.4%)	0/247 (0%)	

Data from HSV seropositive and seronegative patients.
A, Adults (>14 years old); *C*, children (>6 months to 14 years old); *NR*, not reported.
*Health status of patients not identified.

Table III. HSV-1 detection rates in studies that used multiple sampling of healthy individuals

	>1 day and ≤3 weeks		*>3 weeks and ≤7 weeks*	
	Culture	*PCR*	*Culture*	*PCR*
1 or 2 samples weekly or biweekly	ND	ND	37/384 (9.6%) Hatherley et al.[20]	0/14 (0%) Gleeson et al.[44]
3 to 5 samples weekly	6/35 (17.1%) Kaufman et al.[12]	13/61 (21%) Miller et al.[23]	8/418 (1.9%) Lindgren et al.[39]	ND
>5 samples weekly	ND	ND	ND	46/50 (92%) Kaufman[19]
Subtotal	6/35 (17.1%)	13/61 (21.3%)	45/802 (5.6%)	46/64 (71.9%)
Total	19/96 (19.8%)		91/866 (10.5%)	

ND, not done.

oral cavity. Overall, HSV-1 shedding was detected in 6.3% of asymptomatic healthy patients (117 of 1,861) at a single visit, irregardless of seropositivity status. In contrast, HSV-2 shedding in the oral cavity was rare. Only 0.4% of patients (3 of 744) examined at 1 visit shed HSV-2 in the oral cavity.

HSV shedding in the oral cavity at multiple time points. The proportion of individuals positive for HSV-1 shedding was higher in studies that reported multiple-day sampling than the rate reported from single-day sampling. In these studies, HSV-1 was detected in 12.4% of patients (212 of 1,708). In contrast, HSV-2 was detected in 0% (0/247) of patients in the limited number of studies that used multiple-day sampling. Of note, the proportion of patients positive for HSV-1 shedding increased as a function of sampling frequency (Table III). HSV-1 was detected in 10.4% of patients (161 of 1,548) sampled up to 5 times weekly, whereas HSV-1 was detected in 31.9% patients (51 of 160) sampled more than 5 times weekly ($P < .0001$; odds ratio 3.7, 95% confidence interval [CI] 2.5-5.3). Comparison of single- versus multiple-day sampling by the Cochran-Mantel-Haentszel and Breslow-Day statistics showed that asymptomatic HSV-1 shedding was 7.5 times more likely to be detected (95% CI 4.4-12.8; $P < .0001$) than HSV-2 from the oral cavity.

HSV shedding in the oral cavity as a function of time and assay sensitivity. We analyzed the prevalence of oral HSV shedding as a function of time and assay performed, because increasing the sampling frequency can influence the detection rate, and cell culture methods and nucleic acid amplification assays have different levels of sensitivity for the detection of HSV.[18] By cell culture techniques alone, HSV-1 was detected in 115 of 1;528 patients (7.5%) who were evaluated at more than 1 visit (Table III). The rate of daily detection by culture was 1.3% (137 of 10,945) in studies that sampled between 4 and 105 times (median, 60 samplings) over a period of 3 weeks to several months.[8,12,21,22,39,40,44] In contrast, HSV-1 DNA was present in 97 of 180 patients (53.9%) who provided oral samples at multiple visits. The rate of daily detection by polymerase chain reaction (PCR) was 33.3% (1,151 of 3,459) when samples were procured between 3 and 60 times (median, 10 samplings) for 3 weeks up to 11.5 months.

The proportion of patients who shed HSV was highest when samples were procured at least once weekly for at least 3 weeks and assayed by PCR. Under these conditions, HSV-1 was detected in 70.6% of patients (84 of 119). The odds ratio of detecting HSV-1 in samples procured for at least 3 weeks by PCR was 31.01 (95% CI 19.4-46.9; $P < .0001$; Breslow-Day statistic $P = .13$) compared with samples assayed by culture. Of note, in the only study[19] where samples were procured twice daily for 1 month, asymptomatic HSV-1 shedding was detected in the oral cavity of 92% of patients (46 of 50). Interestingly, HSV-1 shedding was observed in HSV seronegative individuals in this[19] and another study.[20]

Duration of shedding. There are a limited number of studies that report the duration of asymptomatic HSV shedding in the oral cavity. In 4 studies that used cell culture assays for the detection of virus, the duration of asymptomatic oral HSV-1 and HSV-2 shedding was similar and reported to be 1 to 1.2 days.[8,16,21,22] The PCR-based studies indicated that the duration of asymptomatic shedding was longer, often for between 1 and 3 days.[19,23] At least one-third of asymptomatic patients shed HSV-1 orally for at least 3 consecutive days once monthly, and a small proportion of patients (up to 10%) orally excreted HSV-1 for between 3 and 7 consecutive days in 1 month.[19,23]

Influence of inflammation and trauma on oral HSV shedding. Inflammation and trauma influence both the frequency and duration of oral HSV shedding. Tateishi et al.[24] reported that 8.0% of patients (26 of 327) with inflammation and 7.2% of patients (5 of 69) with oral

Table III. Continued.

>8 weeks of sampling		
Culture	*PCR*	*Subtotals*
39/553 (7%) Buddingh et al.[13]	13/30 (43%) Knaup et al.[43]; 25/25 (100%) da Silva et al.[28]	114/1006 (11.3%)
8/10 (80%) Douglas et al.[40]; 8/8 (100%) Spruance[8] 4/10 (40%) Okinaga[22]	ND	47/542 (8.7%)
5/110 (4.5%) Kameyama[21]	ND	51/160 (31.9%)
64/691 (9.3%) Total 141/746 (18.9%)	38/55 (69.1%) 212/1708 (12.4%)	

ND, not done.

cysts had HSV-1 in saliva as detected by PCR. This is higher than the overall average of 4.6% (46 of 1,000) of the study cohort. Miller et al.[23] found that the rate of asymptomatic HSV-1 shedding in saliva increased from 8.9% to 14.3% as determined by PCR during the week following a routine dental procedure. Similarly, Hatherley et al.[20] found the rate increased from 2.7% to 13% during the month following dental treatment.

The severity of oral trauma also influences the frequency and duration of oral HSV-1 shedding. Kameyama et al. observed that 11 of 55 patients (20%) undergoing oral surgery and 14 of 83 patients (16.8%) who had orofacial fractures shed HSV-1 in saliva versus 5 of 110 healthy persons (4.5%) not undergoing surgery, as determined by culture of daily saliva specimens collected for up 1 month.[21,25] Further, those with malignant tumors had the highest frequency of shedding (37.5%). In patients who experienced oral trauma (e.g., surgery or orofacial fracture), the mean duration of HSV-1 shedding was 3.7 to 5.8 days.[21,25]

Relation of age and frequency of HSV shedding. Three studies reported the relationship of age and asymptomatic oral HSV excretion. Buddingh et al.[13] first reported that HSV was recovered from saliva more frequently in infants than individuals 15 years or older. He observed that the rate in infants between 7 months and 2 years of age was 20% (15 of 72) and that children between 3 and 14 years of age shed HSV-1 orally at a rate of 9% (18 of 199) whereas individuals 15 years and older had a rate of 2.4% of patient visit days (5 of 185). Tateishi et al.[24] reported that HSV-1 detection by PCR was highest in the age group less than 10 years old (16.7%) compared with those older than 10 years (4.6%; $P < .05$). Miller et al.[26] also found that oral shedding of HSV-1 in dental patients occurred in significantly younger individuals (mean age of expressors 28.1 years) than patients who did not express the virus in the oral cavity (mean age 39.7 years; $P = .02$).

Copy number

Quantitative PCR has been used to measure the amount of viral HSV-1 DNA present in the oral cavity during asymptomatic shedding in 3 studies. In one study,[27] 1 of 58 persons demonstrated HSV-1 DNA in saliva. The viral genome copy number was 20,516/mL of saliva in this patient. In a second larger study of 123 individuals, the mean viral genome copy number was 414,000/mL of saliva of 11 shedders.[26] Levels ranged from 69 to 2,069,895 copies, and the majority (>80%) had greater than 4,000 copies/mL. In a third study,[19] 34% of positive mouth swab specimens (348 of 1,020) contained 240 to 24,000 copies, and 32% of the mouth swabs (325 of 1,020) had greater than 24,000 copies. Together, these data indicate that HSV-1 reactivates frequently and replicates at a subclinical level in the oral cavity of asymptomatic individuals.

DISCUSSION

The present study, which determined the prevalence of asymptomatic HSV shedding in the oral cavity, was undertaken for several reasons. First, there is a growing body of evidence from human studies[11,19,28] indicating that the rate of HSV reactivation and viral shedding is more frequent than previously reported, and a thorough analysis of the current literature was required to clarify the rate of asymptomatic shedding in humans. Second, there are a limited number of reports that have reviewed HSV shedding in the last 10 years,[11,15] and the focus of those reports did not fully address the rate of asymptomatic HSV-1 shedding from the oral cavity or the factors that influence shedding. Third, data from the present study have implications on the interpretation of the dynamics of HSV-1 latency and immune control.[5] And fourth, the implications of HSV shedding have importance with respect to infectivity, risk of transmission, and antiviral control.

The present study analyzed data derived from 22 investigations that evaluated more than 14,000 samples regarding asymptomatic HSV shedding in the oral cavity since 1953. Interpretation of the data is limited in part by the fact that it was not always clear if subjects were asked to report if oral lesions or pain were present or if oral examinations were performed. However, if one assumes that the data were procured as such, the present analyses indicate that important relationships exist between the rate of viral shedding and the viral serotype, specific demographic factors, frequency of sampling, and assay method. The data indicate that: 1)

HSV-1 is more commonly shed orally than HSV-2; 2) asymptomatic HSV-1 shedding in the oral cavity is frequent and episodic; 3) asymptomatic oral HSV-1 shedding is influenced by the age of the individual and recent orofacial trauma and inflammation; and 4) the duration of asymptomatic HSV-1 shedding is limited to a few days in most patients, although oral trauma or inflammation can extend the duration of viral shedding. Finally, the high HSV-1 genome copy numbers observed in the oral cavity supports the prediction that infected saliva is a potential source of transmission.

The predominant HSV shed asymptomatically in the oral cavity of healthy individuals is HSV-1. The odds ratio for detecting HSV-1 versus HSV-2 in saliva and oral mucosal swabs was 7.5. These findings are consistent with reports that asymptomatic HSV-2 shedding from the oral cavity of healthy persons is rare, especially in the absence of symptomatic disease.[11,29,30] The findings reflect the lower seroprevalence of HSV-2 than of HSV-1 in the general population[31] and evidence that transmission of HSV-2 to oral mucosa is less common than that of HSV-1.[26,32,33] It also suggests that viral and site-specific factors are involved in the establishment and/or reactivation phases of HSV latency.

In the present report we found 3 studies that examined the relationship of age and HSV shedding in the oral cavity.[13,24,26] In all 3 studies, younger patients shed HSV-1 more often than older patients. Infants shed HSV-1 more frequently than children, children more frequently than teenagers, and young adults more frequently than middle-aged adults. This may be due to either a reduced efficiency of reactivation in older individuals or those most proximal to the primary infection having higher shedding rates. Consistent with the latter premise, the risk of shedding HSV-2 at genital sites decreases with time subsequent to the primary infection, with persons beyond 10 years from the primary infection having approximately 70% lower shedding rates than during the first 6 months of infection.[34] Although data are lacking on oral HSV-1 shedding in older adults, one can surmise that they are less susceptible for HSV shedding in the oral cavity; however, studies are needed to investigate this possibility. Also, information is needed to determine if oral HSV shedding frequency is altered by recent recurrent HSV infections or oral and/or orogenital contact with active shedders that could theoretically reinoculate the oral mucosa, reseed the trigeminal ganglia, and increase the reservoir of virus for subsequent reactivations.

For over 50 years, the frequency of asymptomatic HSV-1 shedding in the oral cavity has been accepted as occurring in about 6% to 7% of the population.[8,13,15] In fact, that is the rate of occurrence on any given day, as shown in Table I. However, if one analyzes the frequency of detection by the number of samples procured per week (Table III), which is a better measure of shedding patterns, the proportion of persons who shed increases from 6.3% (1 sampling) to 11.4% (1 or 2 samplings per week) to 31.9% (sampling more than 5 times weekly). Further, if one relies only on the more sensitive nucleic acid amplification assays performed on samples procured for at least 3 weeks, then 70% of persons shed HSV-1 in the oral cavity at least once monthly.

Clearly, PCR is 3-4 times more sensitive than cell culture for the detection of HSV shedding[18] and provides a more accurate picture of the prevalence of HSV shedding in the oral cavity. Based on the PCR analyses of da Silva et al.[28] and Kaufman et al.,[19] the frequency of subclinical shedding of HSV-1 varies greatly from one person to another with frequencies ranging from none to 42% of days tested. PCR has also shown that: 1) HSV shedding occurs at many intraoral sites[19,28,35]; 2) asymptomatic shedding (and HSV-1 reactivation) is at least 5 times more common than previously thought; and 3) HSV-1 seropositive persons shed large quantities of virus orally and are likely infectious during asymptomatic periods, because PCR HSV-positive specimens have been demonstrated to be infectious.[18] Also of note, in two studies several antiHSV-1 antibody–negative subjects shed HSV-1 DNA, as determined by viral culture and PCR.[19,20] Because both methods can be more sensitive than immunologic assays for detecting viral antibodies, this finding has implications that the proportion of the general population infected with HSV, as determined by seroprevalence, could be largely underreported.

We observed that the duration of shedding has not changed dramatically from that previously reported.[15] Data are consistent that HSV-1 is shed asymptomatically for 1 to 3 days in healthy individuals. More recent data from Kaufman et al.[19] indicate that most healthy humans shed HSV-1 asymptomatically in the oral cavity for 1 to 2 days for an average of about 13 days per month. Trauma and immunosuppression increase the frequency and duration of shedding.[21,25] Not surprisingly, the more severe the orofacial trauma or immunosuppression, the more likely viral shedding occurs.[36,37]

Several of the findings from the present report have an impact on the practice of dentistry. First, dentists should be aware that HSV shedding in the oral cavity is frequent and common in the absence of oral lesions and that oral secretions commonly contain infectious HSV. Accordingly, dentists are in a unique position to educate patients of the infectious nature of saliva and the risk of transmission during kissing. Also, these data reinforce the importance of implementing proper infec-

tion control measures when performing routine dental examinations and procedures. Efforts should be made to minimize splashes and splatter even in the absence of lesions. Second, medical conditions (e.g., immunosuppression) and traumatic oral procedures increase the likelihood of HSV shedding in the oral cavity. Although it is unclear if these asymptomatic periods of shedding contribute to oral disease, it has been purported that herpesviruses can contribute to the pathogenesis of periodontal disease[35] and persistent mucosal ulcerations.[38] If studies continue to demonstrate important relationships between HSV shedding and specific oral diseases, targeted antiviral therapies may become more commonly used, because they have been shown to reduce both clinical and subclinical reactivation rates of HSV-1[23] and HSV-2.[11,14] Finally, the high frequency of HSV shedding suggests that HSV-1 is not as dormant during latency as previously believed and additional research is required to better understand the mechanisms that regulate reactivation.

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REACTIVATION OF GENITAL HERPES SIMPLEX VIRUS TYPE 2 INFECTION IN ASYMPTOMATIC SEROPOSITIVE PERSONS

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ABSTRACT

Background Most persons who have serologic evidence of infection with herpes simplex virus (HSV) type 2 (HSV-2) are asymptomatic. Historically, it has been assumed that these persons have less frequent viral reactivation than those with symptomatic infection.

Methods We conducted a prospective study to investigate genital shedding of HSV among 53 subjects who had antibodies to HSV-2 but who reported having no history of genital herpes, and we compared their patterns of viral shedding with those in a similar cohort of 90 subjects with symptomatic HSV-2 infection. Genital secretions of the subjects in both groups were sampled daily and cultured for HSV for a median of 94 days.

Results HSV was isolated from the genital mucosa in 38 of the 53 HSV-2–seropositive subjects (72 percent) who reported no history of genital herpes, and HSV DNA was detected by the polymerase-chain-reaction assay in cultures prepared from genital mucosal swabs in 6 additional subjects. The rate of subclinical shedding of HSV in the subjects with no reported history of genital herpes was similar to that in the subjects with such a history (3.0 percent vs. 2.7 percent). Of the 53 subjects who had no reported history of genital herpes, 33 (62 percent) subsequently reported having typical herpetic lesions; the duration of their recurrences in these subjects was shorter (median, three days vs. five days; $P<0.001$) and the frequency lower (median, 3.0 per year vs. 8.2 per year; $P<0.001$) than in the 90 subjects with previously diagnosed symptomatic infection. Only 1 of these 53 subjects had no clinical or virologic evidence of HSV infection.

Conclusions Seropositivity for HSV-2 is associated with viral shedding in the genital tract, even in subjects with no reported history of genital herpes. (N Engl J Med 2000;342:844-50.)

SEROLOGIC surveys indicate that the prevalence of infection with herpes simplex virus (HSV) type 2 (HSV-2) among adults approaches 25 percent in the United States and ranges from 4 to 18 percent in western Europe.[1-5] In most studies, only 10 to 25 percent of subjects with HSV-2 infection report a history of genital lesions.[1,2] Historically, it has been assumed that persons with asymptomatic HSV infections have less frequent and less severe reactivations than those with symptomatic disease. However, two lines of evidence suggest this may not be true. First, many HSV-2–seropositive subjects who initially report having no history of genital lesions do, after an educational session with a clinician, subsequently report having such lesions.[6,7] Such subjects most likely have unrecognized symptomatic infection. Second, most HSV-2 infections are acquired from a person with no history of genital herpes infection.[8,9] These data suggest that viral shedding in seropositive subjects may be frequent, regardless of the presence or absence of a reported history of genital herpes.

To investigate viral shedding in HSV-2–seropositive persons who were asymptomatic, we identified HSV-2–seropositive women and men who reported no history of genital lesions. After they attended an educational session on the symptoms and signs of genital herpes, the subjects were followed with daily cultures of the genital area to evaluate the frequency and the sites of viral shedding in the genital tract. The clinical and virologic characteristics of the infection in these subjects were compared with those in subjects with symptomatic genital HSV-2 infections who followed a similar regimen.[10]

METHODS

Study Subjects

We recruited 37 subjects with no reported history of genital herpes infection from among persons who were identified as being seropositive for HSV-2 in a random screening for HSV antibodies among patients attending a primary care clinic,[11,12] but who reported having no history of genital herpes, and 16 subjects who were recruited as potential candidates for a study of an experimental HSV-2 vaccine but who were unexpectedly found to be positive for HSV-2 antibodies.[13] The study was conducted at the University of Washington Family Medicine Clinic and Virology Research Clinic in Seattle and was approved by the human-subjects review committee of the University of Washington. All subjects gave written informed consent.

All subjects attended an individual standardized educational session on genital herpes that included reviewing photographs of herpetic lesions. Photographs of both typical lesions (e.g., blisters and ulcers) and atypical lesions (e.g., fissures) were shown, and the common symptoms (e.g., itching and tingling) were discussed. The women were taught to obtain swab specimens of the cervicovaginal, vulvar, and perianal areas for viral cultures, as described previously.[10,14] The men were taught to obtain swabs of the penile skin, perianal area, and the urethral meatus or a first-morning urine sample for viral culture.[15-17] The subjects were asked to collect samples daily for three months. The subjects kept a diary of symptoms and

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signs of genital disease and were asked to come to the clinic if they noted lesions consistent with the presence of genital herpes as well as for regular monthly visits.

We also studied 90 subjects (44 men and 46 women) with symptomatic genital herpes who were HSV-2–seropositive and who were attending the Virology Research Clinic in Seattle or Westover Heights Clinic in Portland, Oregon. These subjects attended the same type of educational session and followed the same daily sampling protocol as the 53 HSV-2–seropositive persons with no reported history of genital herpes. The demographic and clinical characteristics of these 90 subjects were similar to those of the large cohort of subjects with symptomatic genital herpes whom we have studied in the past two decades.[18,19]

Laboratory Methods

Serum samples were tested for HSV type 1 (HSV-1) and HSV-2 antibodies by Western blot analysis.[20] Cultures for viral studies were placed in transport medium and delivered to the laboratory three times per week. Viral isolation was performed as previously described; all isolates were confirmed and typed with monoclonal antibodies.[21,22] HSV DNA was measured by a quantitative real-time fluorescence-based polymerase-chain-reaction (PCR) assay.[23]

Study End Points

Herpes lesions were defined as blisters, ulcers, or crusts in the genital or perianal area or on the buttocks. A recurrence was defined as the presence of a lesion at any of these sites on one or more consecutive days. Episodes of viral shedding were defined as the finding of a positive culture on one or more consecutive days. The rate of viral shedding was calculated as the number of days with a positive culture divided by the total number of days on which cultures were obtained. The analysis of subclinical shedding was based only on findings obtained on days on which lesions were absent.

Statistical Analysis

The chi-square and Wilcoxon rank-sum tests were used to compare the characteristics of the 53 subjects who reported having no history of genital herpes with those of the 90 subjects with symptoms. Log-linear models were fitted to examine predictors of viral shedding. Scale factors were estimated to account for greater variability than predicted by the Poisson model.[24] P values for comparisons of the percentage of days with shedding between the subjects with no reported history of genital herpes and the subjects with such a history were obtained from multivariate log-linear models and, hence, are adjusted for age and sex. All statistical tests are two-sided.

RESULTS

Characteristics of the Subjects

The median age of the 53 HSV-2–seropositive subjects (42 women and 11 men) who reported no history of genital herpes was 38 years; 89 percent were white. Twenty of these subjects had antibodies to HSV-2 only, and 33 had antibodies to both HSV-1 and HSV-2. These subjects supplied swabs for viral culture on a median of 98 days. The analysis included 5887 days on which cultures were obtained and more than 17,700 viral cultures from these 53 subjects.

Recognition of Genital Herpes

After education and counseling, 26 of the 42 women (62 percent) and 7 of the 11 men (64 percent) who had reported having no history of genital herpes reported having typical ulcers, blisters, or crusts in the genital area during follow-up; 19 of these 33 subjects reported more than one recurrence of genital herpes. Examples of two symptom diaries are shown in Figure 1. Thirteen subjects never reported lesions but did report localized genital symptoms. In 7 of these 13 subjects, HSV-2 was identified in the genital tract

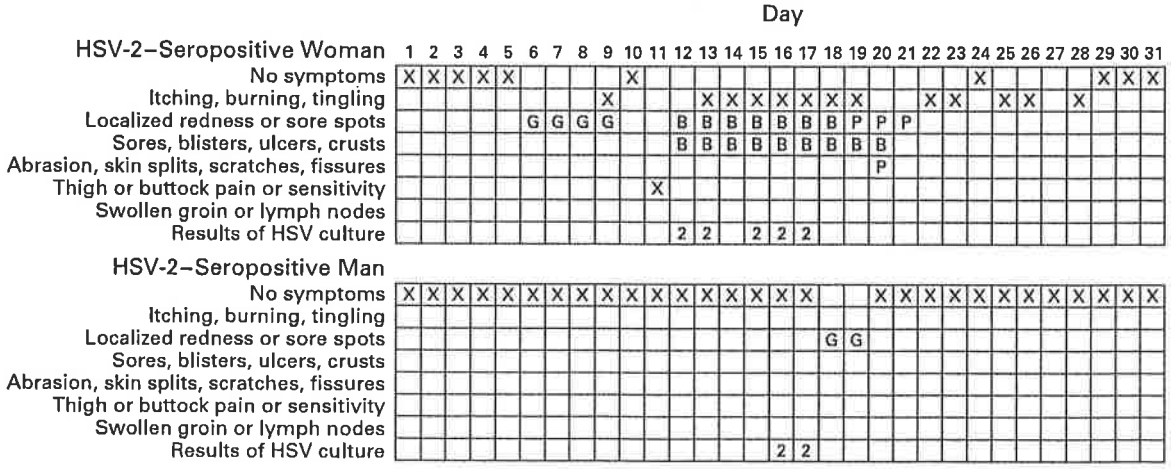

Figure 1. Sample Symptom Diaries Kept by an HSV-2–Seropositive Woman and an HSV-2–Seropositive Man with No Reported History of Genital Herpes.

The woman had antibodies to HSV-1 and HSV-2. During the study, a typical HSV lesion developed on her buttock. During this episode, HSV-2 (2) was isolated initially from the buttock lesion and then from the cervix, vulva, and perianal area. The lesions were located in the genital area (G), buttocks (B), and perianal area (P). The man also had antibodies to HSV-1 and HSV-2. Even after an educational session, the man did not report having typical recurrences. He had an episode of asymptomatic shedding of HSV-2 from the penile skin on days 16 and 17 and mild, nonspecific genital symptoms on days 18 and 19.

TABLE 1. CORRELATION BETWEEN THE RECOGNITION OF GENITAL HERPES LESIONS AND VIRAL SHEDDING IN 53 HSV-2–SEROPOSITIVE SUBJECTS WITH NO REPORTED HISTORY OF GENITAL HERPES.

VARIABLE	HERPES SIMPLEX VIRUS RECOVERED*		
	YES	NO	TOTAL
	no. of subjects		
Genital ulcers, blisters, or crusts	28	5	33
Localized genital itching or soreness	10	3	13
No clinical evidence of genital herpes	6	1	7
Total	44	9	53

*Herpes simplex virus was isolated from culture or identified with use of a polymerase-chain-reaction assay for viral DNA.

when symptoms were present. Thus, 46 of the 53 HSV-2–seropositive subjects (87 percent) who reported no history of genital herpes reported having either genital lesions or localized genital symptoms during follow-up (Table 1).

HSV Shedding in the Genital Area

HSV was isolated by viral culture of swabs from the genital mucosa at least once in 38 of the 53 HSV-2–seropositive subjects (72 percent) who reported no history of genital herpes. HSV-2 was isolated from 37 subjects, and HSV-1 was isolated from 1 woman who was seropositive for both HSV-1 and HSV-2. Of these 38 subjects, 36 had HSV isolated from genital mucosal swabs obtained on days on which lesions were absent (asymptomatic shedding), whereas 18 subjects had HSV isolated on days on which lesions were reported. Among the seven subjects who reported having no symptoms or signs of genital herpes during the study, HSV-2 was isolated from four and HSV-1 from one. Among the 13 subjects who reported only localized genital symptoms but no lesions, HSV-2 was isolated from 9.

Overall, among the 53 HSV-2–seropositive subjects who reported having no history of genital herpes at the time of enrollment, HSV was isolated on 3.8 percent of days on which cultures were obtained (range, 0 to 17 percent). HSV was isolated on 168 of the 5591 days (3.0 percent) on which cultures were obtained in the absence of lesions. Of the 36 subjects who had subclinical viral shedding, HSV was isolated on up to 5 percent of days on which lesions were absent in the case of 22 subjects and on more than 5 percent of days in the case of the remaining 14 subjects (Fig. 2A).

The sensitivity of the PCR assay for the detection

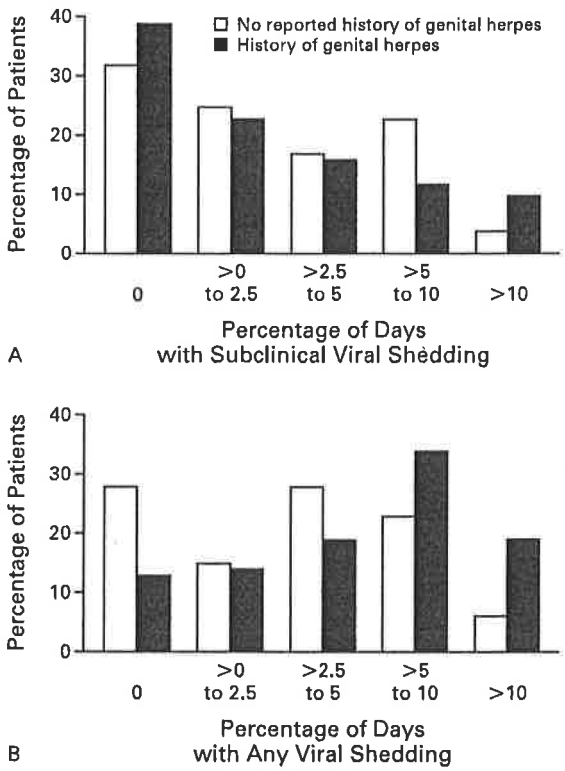

Figure 2. Frequency of Subclinical Viral Shedding (Panel A) and Any Viral Shedding (Panel B) in HSV-2–Seropositive Subjects with No Reported History of Genital Herpes and in Those with Such a History, as Defined by Isolation of the Virus in Tissue Culture.

of HSV is greater than the sensitivity of culture.[23,25] We investigated whether mucosal HSV infection could be demonstrated by the PCR assay in 9 of the 15 subjects from whom HSV was not isolated by standard viral-culture techniques. In six of these nine subjects (four women and two men), HSV DNA was detected by the PCR assay in swabs of genital secretions. The median number of days on which HSV DNA was detected in these six subjects was 3.5 (range, 2 to 11). The geometric mean number of copies of HSV DNA was 8700 per milliliter of PCR specimen (range, 500 per milliliter to 5 million per milliliter). Thus, overall, HSV was detected in genital secretions by either viral culture or PCR assay in 44 of 53 HSV-2–seropositive subjects (83 percent) who reported having no history of genital herpes (Table 1). Only 1 of 53 subjects had no clinical or virologic evidence of HSV infection.

Clinical and Virologic Characteristics in Relation to History and Symptoms

Among the 90 subjects (46 women and 44 men) with a history of genital herpes, the frequency of sero-

TABLE 2. CHARACTERISTICS OF HSV-2–SEROPOSITIVE SUBJECTS WITH NO REPORTED HISTORY OF GENITAL HERPES AND OF THOSE WITH SUCH A HISTORY.

CHARACTERISTIC	NO REPORTED HISTORY OF GENITAL HERPES		HISTORY OF GENITAL HERPES	
	WOMEN (N=42)	MEN (N=11)	WOMEN (N=46)	MEN (N=44)
No. of days on which cultures were obtained				
Median	101	85	82	86
Range	30–219	30–304	34–172	30–375
Viral shedding — no. of subjects (%)	30 (71)	8 (73)	42 (91)	36 (82)
Subclinical viral shedding — no. of subjects (%)	29 (69)	7 (64)	32 (70)	23 (52)
Recurrence — no. of subjects (%)	26 (62)	7 (64)	36 (78)	34 (77)
Viral shedding during a recurrence — no. of subjects (%)	14 (33)	4 (36)	35 (76)	29 (66)
Percentage of days with viral isolation	3.8	3.9	8.3	4.7
Range	0–15	0–17	0–36	0–23
Percentage of days with viral isolation in the absence of lesions	3.0	3.0	3.6	2.0
Range	0–10	0–13	0–35	0–19
Percentage of days with lesions	3.9	4.5	18.0	11.5
Range	0–25	0–25	0–72	0–53
Rate of recurrence per year				
Median	2.8	4.3	8.5	6.6
Range	0–12	0–21	0–30	0–26
Duration of recurrences — days				
Median	3	4	6	5
Range	1–17	1–11	1–53	1–22
Percentage of days with positive cultures without lesions	78	80	35	37

positivity for both HSV-1 and HSV-2 was lower than that among the subjects with no reported history of genital herpes (40 percent vs. 62 percent, P=0.02). The symptomatic subjects were also younger (median age, 33 vs. 38 years; P=0.003). They were predominantly white (92 percent). They supplied swabs for viral culture on a median of 84 days.

The rates of reactivation of HSV infection among the two groups of subjects are shown in Table 2 and Figure 2. The total rate of viral shedding was significantly higher among subjects with a history of genital herpes than among HSV-2–seropositive subjects with no reported history of genital herpes (6.4 percent vs. 3.8 percent of days, P=0.001). Of all the days on which HSV was isolated in each group of subjects, 36 percent were days on which no lesions were reported among subjects with a history of genital herpes, as compared with 79 percent among the subjects with no reported history. However, the rates of subclinical viral shedding (2.7 percent vs. 3.0 percent) (Fig. 2A), the duration of episodes of subclinical shedding (Fig. 3A), and the site-specific rates of subclinical shedding were similar in the group with a history of genital herpes and the group with no reported history.

Among the women, the site-specific rates of subclinical viral shedding were 1.6 percent for the cervicovaginal area for those with a history of genital herpes and 0.9 percent for those with no reported history of genital herpes; 1.4 percent and 1.2 percent, respectively, for the vulvar area; and 1.5 percent and 1.6 percent, respectively, for the perianal area. Among the men, the site-specific rates were 1.1 percent for the penile skin for those with a history of genital herpes and 1.9 percent for those with no reported history and 0.9 percent and 0.7 percent, respectively, for the perianal area.

The recurrence rate was significantly higher among subjects who had a history of genital herpes than among those with no reported history of genital herpes (median, 8.2 per year vs. 3.0 per year; P<0.001) and the recurrences lasted longer (median, five days vs. three days; P<0.001) (Fig. 3B).

In an analysis of risk factors for viral shedding, adjusted for age and sex, the HSV-2–seropositive subjects with a history of genital herpes had a significantly higher risk of viral shedding than those with no reported history (risk ratio, 1.84; 95 percent confidence interval, 1.27 to 2.66) (Table 3). The risk of viral shedding was slightly higher among women than men and tended to decrease with age. In this analysis, age may be serving as a surrogate for the time since the initial infection, which was unknown for subjects with no reported history of genital herpes, because the rates of viral shedding decrease over time among

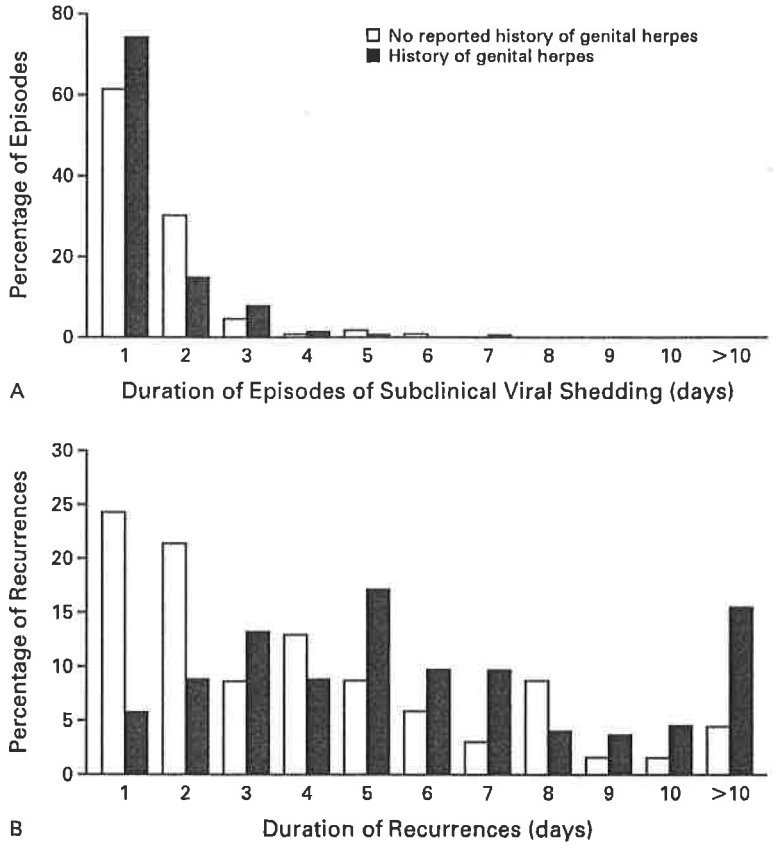

Figure 3. Duration of Episodes of Subclinical Shedding (Panel A) and Recurrences of Genital Herpes (Panel B) in Subjects with No Reported History of Genital Herpes and in Those with Such a History.

symptomatic subjects.[10,26] In contrast to the risk of any viral shedding, the adjusted risk of subclinical shedding in the subjects with a history of genital herpes did not differ significantly from those with no reported history of genital herpes (risk ratio, 0.95; 95 percent confidence interval, 0.59 to 1.53).

DISCUSSION

There has been controversy regarding the biologic and clinical meaning of asymptomatic HSV-2 infection. At present, the medical and public health communities largely ignore persons who have asymptomatic HSV-2 infection because little information is available regarding the benefit of identifying such persons. We systematically evaluated the rates of reactivation of infection among asymptomatic HSV-2–seropositive subjects. We found that 83 percent of subjects who were HSV-2–seropositive but who reported having no history of genital lesions had genital shedding of HSV during follow-up. Although much of the shedding was subclinical, once identified as seropositive by serologic testing and educated about their HSV-2 infection, 62 percent of such subjects reported having typical herpetic lesions. The pattern, sites, and frequency of subclinical reactivation of infection in these subjects were similar to those in subjects with symptomatic infections.

In this study, as in prior studies,[6,7] knowledge of their seropositivity for HSV-2 combined with education regarding the clinical manifestations of genital herpes resulted in the recognition of typical lesions among most subjects with "silent" HSV-2 infections. The subjects' lack of recognition of recurrent genital herpes may be explained by the lower frequency and shorter duration of lesions among subjects with silent infections, as compared with those with clinically evident genital herpes.[10] Although perception clearly has an important role in the diagnosis of this disease, the reported frequency or duration of episodes noted by the subjects was unlikely to have been underestimated, given the intensive follow-up and frequent visits required by our protocol. Subjects with symptomatic

TABLE 3. PREDICTORS OF VIRAL SHEDDING, AS MEASURED BY VIRAL CULTURE, IN SUBJECTS WITH HSV-2 INFECTION.*

CHARACTERISTIC	UNIVARIATE RISK RATIO (95% CI)	ADJUSTED RISK RATIO (95% CI)†
Any viral shedding		
History of genital herpes (vs. no reported history)	1.70 (1.17–2.46)	1.84 (1.27–2.66)
Women (vs. men)	1.30 (0.90–1.87)	1.43 (0.99–2.06)
Age (per year of age)	0.98 (0.96–0.99)	0.99 (0.97–1.00)
Seropositivity for HSV-1 and HSV-2 (vs. HSV-2 alone)‡	0.90 (0.63–1.28)	—
Subclinical shedding		
History of genital herpes (vs. no reported history)	0.90 (0.57–1.42)	0.95 (0.59–1.53)
Women (vs. men)	1.48 (0.91–2.39)	1.33 (0.79–2.25)
Age (per year of age)	0.98 (0.96–1.00)	0.98 (0.96–1.01)
Seropositivity for HSV-1 and HSV-2 (vs. HSV-2 alone)‡	0.99 (0.63–1.56)	—

*CI denotes confidence interval.

†The risk ratios have been adjusted for age and sex.

‡The difference between seropositivity for both HSV-1 and HSV-2 and seropositivity for HSV-2 alone was not significant in any model, so it was omitted from the multivariate models used to obtain adjusted risk ratios.

infection typical of those enrolled in treatment trials are at the more severe end of the clinical spectrum of HSV-2 infection.[27-29]

Whether host or viral factors are responsible for the difference in clinical and virologic manifestations of infection between subjects with frequent reactivation of HSV and those with infrequent reactivation is not known. However, the rates of subclinical viral shedding were similar among the subjects with previously unrecognized genital herpes and those with recognized infection. HSV is often transmitted during episodes of subclinical shedding[8,30]; with regard to infectivity, HSV-2–seropositive persons who initially reported having no lesions differed little from HSV-2–seropositive persons who recognized the lesions. Our findings concerning the potential for the transmission of HSV to sexual partners are therefore not comforting to either patients or providers.

Asymptomatic shedding of HSV has been investigated predominantly among women. Because women may shed virus "internally" (i.e., from the cervix and vagina), the idea that some reactivations of infection may go unnoticed appears plausible. In contrast, the anatomy of the genital tract in men and the fact that the genital epithelium is predominantly skin and not mucosa have made less plausible the concept of asymptomatic shedding from men's genital skin. This issue was illustrated in a study of physicians' attitudes toward asymptomatic shedding of HSV,[31] in which both male and female physicians tended to dismiss the possibility of asymptomatic shedding in men as anatomically implausible. We found that this reasoning is erroneous; the rates of subclinical shedding among men approximated those among women.

Although our study included more than 17,700 viral cultures from HSV-2–seropositive persons with no history of genital herpes, all cultures were obtained from a total of 53 such subjects. Since studies such as ours are difficult and time consuming for the subjects, only subjects who were particularly concerned about HSV-2 infection and who were able to comply with the procedures participated. However, since most of the asymptomatic subjects in this study were initially recruited in a general medical clinic and did not present with genitourinary symptoms, it seems unlikely that the cohort was biased toward those with more severe subclinical HSV infections.

Prevention of the spread of HSV to neonates and sexual partners will require the identification and control of infection in persons with subclinical HSV infection. Although accurate, type-specific serologic tests have been available in research laboratories for more than a decade, commercially available assays have been developed and marketed only fairly recently.[32-36] Thus, it is just becoming possible to identify the large reservoir of persons with infrequent, short episodes of HSV-2 reactivation. Our data suggest that such persons often may not require antiviral chemotherapy for clinical symptoms and signs of infection because their episodes are short and infrequent. However, they do require education and counseling regarding their risk of transmitting the infection to others.

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FARLEY

EXHIBIT B

ORIGINAL CONTRIBUTION

Trends in Herpes Simplex Virus Type 1 and Type 2 Seroprevalence in the United States

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Context Herpes simplex virus type 1 (HSV-1) and type 2 are common infections worldwide. Herpes simplex virus type 2 (HSV-2) is the cause of most genital herpes and is almost always sexually transmitted. In contrast, HSV-1 is usually transmitted during childhood via nonsexual contacts. Preexisting HSV-1 antibodies can alleviate clinical manifestations of subsequently acquired HSV-2. Furthermore, HSV-1 has become an important cause of genital herpes in some developed countries.

Objective To examine trends in HSV-1 and HSV-2 seroprevalence in the United States in 1999-2004 compared with 1988-1994.

Design, Settings, and Participants Cross-sectional, nationally representative surveys (US National Health and Nutrition Examination Surveys [NHANES]), were used to compare national seroprevalence estimates from 1999-2004 with those from 1988-1994, and changes in HSV-1 and HSV-2 seroprevalence since 1976-1980 were reviewed. Persons aged 14 to 49 years were included in these analyses.

Main Outcome Measures Seroprevalence of HSV-1 and HSV-2 antibodies based on results from type-specific immunodot assays; diagnosis of genital herpes.

Results The overall age-adjusted HSV-2 seroprevalence was 17.0% (95% confidence interval [CI], 15.8%-18.3%) in 1999-2004 and 21.0% (95% CI, 19.1%-23.1%) in 1988-1994, a relative decrease of 19.0% between the 2 surveys (95% CI, −28.6% to −9.5%; $P<.001$). Decreases in HSV-2 seroprevalence were especially concentrated in persons aged 14 to 19 years between 1988 and 2004. In adolescents aged 17 to 19 years and young adults, the decreases in HSV-2 seroprevalence were significant even after adjusting for changes in sexual behaviors. Among those infected with HSV-2, the percentage who reported having been diagnosed with genital herpes was statistically different (14.3% in 1999-2004 and 9.9% in 1988-1994; $P=.02$). Seroprevalence of HSV-1 decreased from 62.0% (95% CI, 59.6%-64.6%) in 1988-1994 to 57.7% (95% CI, 55.9%-59.5%) in 1999-2004, a relative decrease of 6.9% between the 2 surveys (95% CI, −11.6% to −2.3%; $P=.006$). Among persons infected with HSV-1 but not with HSV-2, a higher percentage reported having been diagnosed with genital herpes in 1999-2004 compared with 1988-1994 (1.8% vs 0.4%, respectively; $P<.001$).

Conclusions These data show declines in HSV-2 seroprevalence, suggesting that the trajectory of increasing HSV-2 seroprevalence in the United States has been reversed. Seroprevalence of HSV-1 decreased but the incidence of genital herpes caused by HSV-1 may be increasing.

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HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) is the cause of most genital herpes and is one of the most prevalent sexually transmitted infections worldwide.[1-3] Herpes simplex virus type 1 (HSV-1) is typically transmitted during childhood via nonsexual contact.[4] Most HSV-1 and HSV-2 infections are subclinical. When infection is symptomatic, the clinical manifestations of HSV-2 are typically characterized by recurrent, painful vesicular and ulcerative lesions in the genital and anal areas.[3-5] In contrast, symptomatic HSV-1 infections are usually manifested as recurrent orolabial and facial lesions.[3] However, HSV-1 has emerged as a principle causative agent of genital herpes in some developed countries.[6-9] In the United States, HSV-1 is an important cause of genital herpes and its importance is increasing in college students and other selected populations.[10-13] Both HSV-1 and HSV-2 can also cause infrequent but serious diseases such as blindness, encephalitis, and neonatal infections.[4]

Strong synergy has been found between HSV-2 and human immunodeficiency virus (HIV).[14-17] Infection with HSV-2 can at least double the risk for sexually acquired HIV infection because recurrent genital herpes can provide a port of entry for HIV and recruit HIV target cells to the sites of epithelial infection.[18,19] Infection with HSV-2 may accelerate HIV progression and increase the infectiousness of HIV, thus enhancing sexual transmission of HIV.[20-22] Monitoring HSV-2 seroprevalence may help direct HIV prevention efforts to populations at greater risk of acquiring or transmitting HIV infection.

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Changes in HSV-1 seroprevalence can alter the clinical manifestations of subsequently acquired HSV-2 infection. Compared with persons seropositive for HSV-1, those persons lacking HSV-1 antibodies are almost 3 times more likely to have a symptomatic HSV-2 infection.[13,23] Seroprevalence of HSV-1 may also influence decisions about HSV-2 vaccination strategies because the vaccine that is being clinically tested may be efficacious only in those seronegative for HSV-1.[24]

Serosurveys have been one of the best approaches to study the epidemiology of HSV infections. In the United States, data from National Health and Nutrition Examination Surveys (NHANES) during 1976-1980 (NHANES II) and 1988-1994 (NHANES III) indicated that the overall seroprevalence of HSV-2 increased by 30%,[25] while the overall HSV-1 seroprevalence was unchanged.[26] We examined national trends in HSV-1 and HSV-2 seroprevalence in the 1999-2004 survey compared with the 1988-1994 survey.

METHODS

Study Population and Survey Design

The NHANES are a series of cross-sectional national surveys conducted by the National Center for Health Statistics. Details of the survey methods have been published previously.[27] Briefly, during each survey, a random sample of the US civilian, noninstitutionalized population was selected using a complex, stratified, multistage probability sample design. Some populations, such as adolescents, non-Hispanic blacks, and Mexican Americans were oversampled. Persons selected for the surveys were interviewed and underwent a health examination. In 1999, the NHANES was redesigned to become a continuous survey using otherwise similar methods. A nationally representative sample of the US civilian, noninstitutionalized population is selected each year and usually data from 2 or more years are combined to achieve adequate sample sizes for analyses. Seroprevalence of HSV has been part of NHANES since 1988-1994 but national HSV seroprevalence was first estimated using leftover sera samples from 1976-1980.

For 1999-2004, *sex* was defined as vaginal, oral, or anal. In contrast, the term *sexual intercourse* was used in 1988-1994. In addition, questionnaires were administered using audio computer-assisted self-interview in 1999-2004 instead of face-to-face interview in 1988-1994. A question about history of diagnosed genital herpes was asked for persons aged 18 years or older ("Has a doctor or other health care professional ever told you that you had genital herpes?").

Our analyses focused on the trends in HSV-1 and HSV-2 seroprevalence between 1988-1994 and 1999-2004. About 10.5 years separates the midpoint between the 2 surveys. The common age groups for the surveys are persons aged 14 to 49 years. Of the persons aged 14 to 49 years who were selected for the NHANES in 1988-1994, 84% were interviewed, 78% were examined, and 61% were tested for HSV-1 and HSV-2. For 1999-2004, the corresponding numbers were 82%, 78%, and 72%, respectively.

The NHANES survey for 1999-2004 was approved by the institutional review board of the US Centers for Disease Control and Prevention. Informed consent was obtained from survey participants or their legal guardians.

Laboratory Methods

Purified glycoproteins specific for HSV-1 (gG-1) or HSV-2 (gG-2) were used as antigens to detect type-specific antibodies using solid-phase enzymatic immunodot assays.[28,29] The performance of the immunodot assays is high with respect to sensitivity and ability to discriminate between HSV-1 and HSV-2.[28-30] In 1999-2004, the same immunodot assays and the same laboratory were used as for previous NHANES.[25,26]

Statistical Analyses

SUDAAN software version 9.0 (Research Triangle Institute, Cary, NC) was used for statistical analyses to account for the complex survey design. We estimated HSV-1 and HSV-2 seroprevalence by age, sex, and race/ethnicity according to NHANES design domains. Confidence intervals (CIs) for the seroprevalence estimates were calculated based on a log transformation with the SE calculated using the Δ method.[31] Differences in seroprevalence between the surveys were considered to be statistically significant if the 2-sampled t test had a P value of less than .05.[32]

Race/ethnicity categories were nearly identical between 1988-1994 and 1999-2004[33] and were defined by self-report as non-Hispanic black, non-Hispanic white, and Mexican American. Persons who did not fit into these categories were classified as *other* and were included in the total population. The race categories used for 1976-1980 were white, black, and other. To permit comparisons between the 3 surveys, participants who reported Hispanic ancestry in the 1976-1980 survey were excluded from the white and black race categories.

All seroprevalence estimates were weighted to represent the US civilian, noninstitutionalized population and to account for oversampling and nonresponse to the interview and the medical examination.[34] Among participants aged 14 to 49 years who were interviewed and examined during 1999-2004, 8.6% did not have HSV serological results. The reasons for missing results may include refusal or unsuccessful venipuncture or the need to use serum for other tests. We investigated the impact of the missing serological results on HSV-1 and HSV-2 seroprevalence estimates by identifying significant demographic predictors of missing results through a weighted logistic regression model and used the model to further adjust the weights. The estimates using further adjusted weights changed slightly (changes in point estimates ranging from −1.3% to 0.6% for HSV-1 and −0.1 to 0.9% for HSV-2) but were within 95% CIs of seroprevalence estimates based on weights published by the National

Center for Health Statistics. Similar results were found from investigations into the missing HSV serological results in 1988-1994. These results convinced us to use weights from the National Center for Health Statistics in our analyses.

Logistic regression methods described by Korn and Barry[35] were used to examine whether differences in sociodemographic or sexual behavior between the survey participants might explain the observed changes in HSV-2 seroprevalence between 1988-1994 and 1999-2004. The survey indicator variable was forced into the model, and other variables were added in order of statistical significance. The criteria for inclusion and remaining in the model was based on the Satterthwaite-adjusted F-test P value of .10 or less. Once all variables that met the criteria had been included in the model, pairwise interactions were evaluated. Several significant interactions with sex led us to develop models separately for males and females. Variables that met the entry criteria in either model were included in the final models for both sexes. To facilitate interpretations, adjusted seroprevalence was calculated from the sex-specific logistic regression model using the PREDMARG statement in SUDAAN. The adjusted seroprevalence, also known as predictive margin, was generated using the logistic regression model to estimate the probability of being HSV-2 positive for every individual, averaging over the distribution of the covariates among the entire weighted sample.

Table 1. Weighted Herpes Simplex Virus 2 Seroprevalence for NHANES in 1999-2004

	Sample Size	HSV-2 Seroprevalence (95% CI)	*P* Value*
Overall	11 508	17.2 (15.9-18.7)	
Sex			
Male	5511	11.2 (9.9-12.8)	<.001
Female	5997	23:1 (21.5-24.9)	
Race/ethnicity			
Non-Hispanic white	4311	13.7 (12.5-15.0)	<.001
Non-Hispanic black	2926	40.3 (37.3-43.5)	
Mexican American	3406	11.9 (10.4-13.5)	
Other†	865	17.7 (14.2-22.1)	
Age group, y			
14-19	4650	1.6 (1.3-2.0)	<.001
20-29	2412	10.6 (8.9-12.5)	
30-39	2251	22.1 (20.1-24.3)	
40-49	2195	26.3 (24.2-28.7)	
Marital status			
Never married	6154	10.3 (8.8-12.0)	<.001
Living with partner	682	24.9 (20.9-29.6)	
Married	3595	16.8 (15.1-18.7)	
Divorced	486	35.9 (29.8-43.2)	
Separated	274	34.5 (28.5-41.9)	
Widowed	48	47.4 (27.1-83.0)	
Poverty index			
<Poverty level	2732	21.5 (18.7-24.7)	<.001
≥Poverty level	7909	16.5 (15.2-17.9)	
Education			
<High school	5614	15.8 (14.0-17.7)	.14
High school	2211	17.7 (15.0-20.8)	
>High school	3675	17.8 (16.1-19.6)	
Ever used cocaine‡			
Yes	1052	28.6 (25.4-32.3)	<.001
No	5911	14.7 (13.1-16.5)	
Age at first sex, y§			
≤17	5788	21.1 (19.5-22.9)	<.001
≥18	2405	14.3 (12.6-16.3)	
Lifetime No. of sex partners			
0	2342	2.6 (1.4-5.0)‖	<.001
1	1568	3.8 (2.6-5.7)	
2-4	2432	13.3 (11.4-15.6)	
5-9	1843	20.8 (18.5-23.5)	
10-49	1847	27.2 (25.0-29.6)	
≥50	284	39.9 (33.7-47.3)	

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.
*Calculated using χ^2 test.
†Includes all participants who do not belong to the 3 main racial/ethnic groups, such as those whose race/ethnicity was missing and persons who reported "multiracial."
‡Estimates from NHANES for 1999-2002 only. Drug use data have not been released from NHANES for 2003-2004.
§Defined as vaginal, oral, or anal.
‖Estimates may be unreliable because the relative SE is large (SE/seroprevalence >30%).

RESULTS

Seroprevalence of HSV-2 in 1999-2004

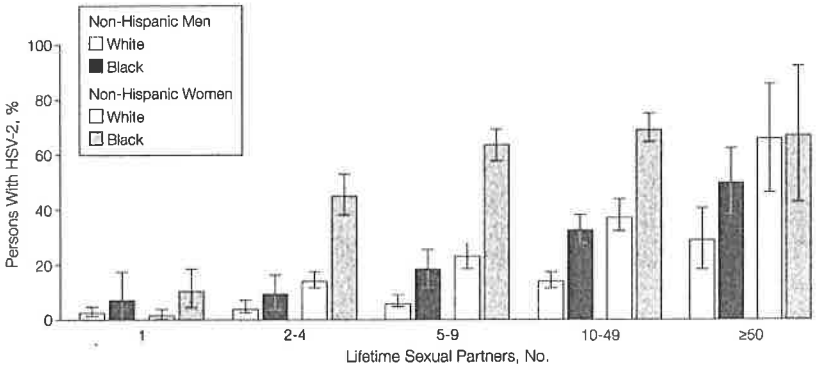
In 1999-2004, the seroprevalence of HSV-2 among participants aged 14 to 49 years was 17.2% (95% CI, 15.9%-18.7%; TABLE 1). The seroprevalence was higher among females than among males (23.1% vs 11.2%; $P<.001$). Seroprevalence of HSV-2 was 13.7% among non-Hispanic whites, 40.3% among non-Hispanic blacks, and 11.9% among Mexican Americans.

The overall HSV-2 seroprevalence increased rapidly with increasing age from 1.6% in participants aged 14 to 19 years to 26.3% in participants aged 40 to 49 years.

As reported in previous NHANES,[25] HSV-2 seroprevalence also varied significantly by a number of demographic and behavioral factors (Table 1). Seroprevalence of HSV-2 was higher among persons who were divorced, separated, or widowed; those living below the poverty level; who had ever used cocaine; and who had sex for the

first time at the age of 17 years or younger. Seroprevalence of HSV-2 was 3.8% in those who reported 1 lifetime sex partner. This prevalence increased to 39.9% in those who reported 50 or more lifetime partners. Seroprevalence of HSV-2 was higher in those who had a larger number of lifetime sexual partners (FIGURE 1). In non-Hispanic black women, however, the seroprevalence of HSV-2 was significantly higher compared with others who reported the same number of lifetime partners.

Figure 1. Age-Adjusted Herpes Simplex Virus Type 2 Seroprevalence According to the Lifetime Number of Sex Partners, by Race/Ethnicity and Sex on NHANES in 1999-2004



Error bars indicate 95% confidence intervals; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.

Trends in HSV-2 Seroprevalence Between 1988-1994 and 1999-2004

The analyses included 9165 persons from the 1988-1994 survey and 11 508 persons from the 1999-2004 survey who were aged 14 to 49 years and had serum samples tested for HSV-2 (TABLE 2). Using the 2000 US Census civilian, noninstitutionalized population aged 14 to 49 years as the standard, the overall age-adjusted HSV-2 seroprevalence was 21.0% (95% CI, 19.1%-23.1%) in 1988-1994 and 17.0% (95% CI, 15.8%-18.3%) in 1999-2004, a relative decrease of 19.0% (95% CI, −28.6% to −9.5%) between the 2 surveys ($P<.001$). Seroprevalence of HSV-2 decreased significantly in males, non-Hispanic whites, and Mexican Americans. The overall decreases in females and non-Hispanic blacks were not statistically significant.

The decrease in HSV-2 seroprevalence was concentrated in the younger age groups. The decreases were significant in all age groups except in participants aged 40 to 49 years (Table 2). Statistically significant decreases occurred in adolescents aged 14 to 19 years in both males and females and in all race/ethnicity groups. Based on the civilian, noninstitutionalized population counts from the 2000 US Census, there were 24 million adolescents aged 14 to 19 years in the United States; the decrease in HSV-2 seroprevalence from 5.8% to 1.6% corresponds to 1 million fewer infections in this age group alone.

Selected sexual behaviors between 1988-1994 and 1999-2004 were compared. Due to different ages at which sexual behavior questions were asked in the 2 surveys, the percentage who ever had sex were compared only in those aged 15 years or older and the number of lifetime sex partners were compared only in those aged 17 years or older. The percentage of participants aged 15 to 19 years who reported having had sex decreased from 59.6% in 1988-1994 to 54.8% in 1999-2004, a relative decrease of 8% ($P=.05$); among participants aged 20 to 29 years, the decrease was 4% from 95.5% to 91.6% ($P=.002$). Among those who had sex, the mean number of lifetime sex partners did not differ in participants aged 17 to 19 years but increased in those aged 20 to 49 years ($P<.001$). Further analyses showed that this increase occurred only in females and was statistically significant in all 3 age groups from 20 through 49 years. The geometric mean number of sex partners in females aged 20 to 49 years increased from 3.3 (95% CI, 3.1-3.6) in 1988-1994 to 4.5 (95% CI, 4.2-4.7) ($P<.001$).

The adjusted HSV-2 seroprevalence in males and females after controlling for differences in sociodemographics and/or sexual behaviors between the 2 surveys are presented in TABLE 3. The decrease in HSV-2 seroprevalence became significant in females but the overall decreases were still greater in males (31.9%) than in females (19.4%) (Table 3). Adjusted HSV-2 seroprevalence significantly decreased in males younger than 40 years and in all female age groups with the exception of those aged 30 to 39 years. In males, the adjusted decreases were significant in non-Hispanic whites and Mexican Americans. In females, the adjusted decreases were significant among all 3 racial/ethnic groups.

Trends in HSV-1 Seroprevalence Between 1988-1994 and 1999-2004

In 1999-2004, HSV-1 seroprevalence varied by age and race/ethnicity, similar to the previous report.[26] The overall age-adjusted seroprevalence of HSV-1 was 62.0% (95% CI, 59.6%-64.6%) in 1988-1994 and 57.7% (95% CI, 55.9%-59.5%) in 1999-2004, a relative decrease of 6.9% (95% CI, −11.6% to −2.3%; $P=.006$; TABLE 4). When HSV-1 seroprevalence was compared by key demographic variables, changes ranging from −21.6% to 2.6% were observed (Table 4). Although the decreases were statistically significant in many groups, there was no concentration in any subpopulation defined by age, sex, or race/ethnicity.

Further analyses limited only to persons born in any of the 50 states in the United States or in Washington, DC,

were performed because birthplace can be an important determinant for HSV-1 acquisition during childhood. The corresponding age-adjusted seroprevalence was generally lower in persons born in the United States but the trend was similar to that in the total population. In US-born persons, HSV-1 seroprevalence decreased by 10% from 59.4% (95% CI, 56.8%-62.0%) in 1988-1994 to 53.3% (95% CI, 51.3%-55.4%) in 1999-2004 ($P<.001$). As in the total US population, the decreases in HSV-1 seroprevalence in those born in the United States did not show a concentration in any subpopulations.

Trends in HSV-1 and HSV-2 Coinfection Between 1988-1994 and 1999-2004

The seroprevalence of coinfection with HSV-1 and HSV-2 decreased from 14.6% in 1988-1994 to 10.5% in 1999-2004 ($P<.001$). In contrast, the seroprevalence of HSV-2 only did not

Table 2. Changes in Weighted Herpes Simplex Virus 2 Seroprevalence in Persons Aged 14 to 49 Years Between NHANES in 1988-1994 and 1999-2004

	NHANES				
	1988-1994		1999-2004		
	Sample Size	HSV-2 Seroprevalence, % (95% CI)	Sample Size	HSV-2 Seroprevalence, % (95% CI)	Change, % (95% CI)
Overall*	9165	21.0 (19.1 to 23.1)	11 508	17.0 (15.8 to 18.3)	−19.0 (−28.6 to −9.5)†
Age group, y					
14-19	1787	5.8 (4.4 to 7.5)	4650	1.6 (1.3 to 2.0)	−72.4 (−81.5 to −63.3)†
20-29	2750	17.2 (14.9 to 19.8)	2412	10.6 (8.9 to 12.5)	−38.4 (−51.6 to −25.2)†
30-39	2567	27.8 (24.6 to 31.4)	2251	22.1 (20.1 to 24.3)	−20.5 (−32.4 to −8.6)†
40-49	2061	26.3 (23.1 to 30.1)	2195	26.4 (24.3 to 28.7)	0 (−15.3 to 15.3)
Sex by age group, y					
Male					
All ages*	4422	17.0 (14.6 to 19.7)	5511	11.2 (9.9 to 12.6)	−34.1 (−46.4 to −21.9)†
14-19	847	5.6 (3.9 to 8.0)	2368	0.9 (0.5 to 1.5)	−83.9 (−93.7 to −74.2)†
20-29	1332	12.1 (9.0 to 16.1)	1044	5.6 (4.0 to 7.9)	−53.7 (−73.9 to −33.5)†
30-39	1203	24.4 (20.5 to 29.1)	1005	14.5 (12.6 to 16.7)	−40.6 (−53.6 to −27.6)†
40-49	1040	20.2 (16.1 to 25.4)	1094	18.6 (15.7 to 21.9)	−7.9 (−33.3 to 17.4)
Female					
All ages*	4743	25.2 (23.2 to 27.3)	5997	22.8 (21.2 to 24.4)	−9.9 (−19.3 to 0)
14-19	940	5.9 (4.5 to 7.9)	2282	2.3 (1.7 to 3.2)	−61.0 (−77.4 to −44.6)†
20-29	1418	22.3 (19.5 to 25.5)	1368	15.6 (13.1 to 18.5)	−30.0 (−44.9 to −15.2)†
30-39	1364	31.2 (27.8 to 35.0)	1246	29.5 (26.6 to 32.7)	−5.4 (−19.7 to 8.8)
40-49	1021	32.6 (28.1 to 37.8)	1101	33.9 (31.1 to 37.1)	3.7 (−13.8 to 21.2)
Race/ethnicity by age group, y‡					
Non-Hispanic white					
All ages*	2652	16.5 (14.4 to 18.9)	4311	13.0 (12.0 to 14.1)	−21.2 (−33.2 to −9.2)§
14-19	461	4.0 (2.6 to 6.4)	1220	1.0 (0.6 to 1.7)	−75.0 (−91.5 to −58.5)†
20-29	675	14.7 (11.8 to 18.2)	1042	6.4 (4.8 to 8.5)	−56.5 (−71.5 to −41.5)†
30-39	792	21.8 (18.5 to 25.8)	1070	18.0 (15.5 to 20.7)	−17.4 (−35.0 to 0.1)
40-49	724	19.6 (16.0 to 24.0)	979	20.7 (18.9 to 22.8)	5.6 (−17.1 to 28.3)
Non-Hispanic black					
All ages*	3007	43.2 (41.2 to 45.3)	2926	41.7 (38.5 to 45.1)	−3.9 (−12.5 to 4.7)
14-19	598	11.2 (8.0 to 15.5)	1470	5.0 (3.8 to 6.6)	−55.4 (−73.5 to −37.2)†
20-29	891	33.3 (29.9 to 37.1)	476	35.3 (29.9 to 41.8)	6.0 (−14.3 to 26.3)
30-39	884	54.2 (50.2 to 58.6)	460	53.5 (48.8 to 58.6)	−1.5 (−12.8 to 9.9)
40-49	634	59.0 (55.2 to 63.1)	520	56.2 (50.8 to 62.1)	−5.4 (−16.6 to 5.8)
Mexican American					
All ages*	3113	22.6 (20.4 to 25.0)	3406	13.6 (12.1 to 15.3)	−39.8 (−48.8 to −30.8)†
14-19	636	5.7 (3.9 to 8.4)	1658	0.9 (0.5 to 1.5)	−84.2 (−93.7 to −74.7)†
20-29	1072	14.8 (12.5 to 17.6)	678	7.9 (5.3 to 11.7)	−46.6 (−68.6 to −24.7)†
30-39	793	28.8 (26.2 to 31.8)	505	14.2 (11.2 to 18.0)	−51.0 (−63.0 to −39.1)†
40-49	612	32.8 (27.9 to 38.5)	565	25.2 (20.9 to 30.4)	−23.2 (−41.2 to −5.1)§

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.
*Age-adjusted using the 2000 US Census civilian, noninstitutionalized population aged 14 to 49 years as the standard.
†$P<.005$.
‡For the 1988-1994 survey, 393 persons in the "other" race/ethnicity category were excluded; for the 1999-2004 survey, 865 persons in the "other" race/ethnicity category were excluded.
§$P<.05$.

change (6.4% in 1988-1994 and 6.7% in 1999-2004). As a result, the percentage of HSV-2 infected persons who lacked HSV-1 antibodies increased from 30.4% in 1988-1994 to 38.9% in 1999-2004 ($P=.002$). There were no changes in the overall seroprevalence of HSV-1 only. However, the percentage of participants seronegative for both HSV-1 and HSV-2 increased from 32.0% in 1988-1994 to 35.4% in 1999-2004 ($P=.02$). Among adolescents aged 14 to 19 years, the percentage of participants seronegative for both HSV-1 and HSV-2 increased from 52.8% in 1988-1994 to 60.2% in 1999-2004 ($P=.002$).

Trends in the Diagnosis of Genital Herpes

The overall percentage of survey participants who reported having been diagnosed with genital herpes did not change significantly between 1988-1994 and 1999-2004 (3.3% and 3.8%, respectively). However, among those who were HSV-2 seropositive, the percentage reporting a diagnosis of genital herpes increased from 9.9% in 1988-1994 to 14.3% in 1999-2004 ($P=.02$). The percentage of persons having been diagnosed with genital herpes increased among those infected with HSV-2 only and among those with both HSV-1 and HSV-2 but the differences between the 2 surveys did not reach statistical significance (FIGURE 2). In both 1988-1994 and 1999-2004, persons infected with HSV-2 only were significantly more likely to report having been diagnosed with genital herpes than those infected with both HSV-1 and HSV-2. In 1988-1994, 6.6% of those infected with both HSV-1 and HSV-2 had been diagnosed with genital herpes compared with 17.2% in those infected with HSV-2 only ($P=.001$; Figure 2). Similarly, in 1999-2004, 11.0% of those infected with both HSV-1 and HSV-2 had been diagnosed with genital herpes compared with 19.3% in those infected with HSV-2 only ($P<.001$).

A higher percentage of persons infected with HSV-1 only reported having been diagnosed with genital herpes in 1999-2004 than in 1988-1994 (1.8% vs 0.4%; $P<.001$) (Figure 2). Furthermore, in 1999-2004, a person infected with HSV-1 only was more likely to have been diagnosed with genital herpes compared with those seronegative for HSV-1 (adjusted odds ratio, 2.9; 95% CI, 1.7-5.0), while there was no difference in 1988-1994. The mean age for persons infected with HSV-1 only who had been diagnosed with genital herpes was similar between 1988-1994 and 1999-2004.

Review of HSV-1 and HSV-2 Seroprevalence from 3 NHANES

In FIGURE 3, HSV-2 seroprevalence from the 3 NHANES are presented for non-Hispanic whites and non-Hispanic blacks by age. No data were available for Mexican Americans in 1976-1980. In non-Hispanic whites, HSV-2 seroprevalence from the 1999-2004 survey was not significantly different from that observed in 1976-1980 for any of the age groups. This indicates that the significant increases among participants aged 14 to 19 years, 20 to 29 years, and 30 to 39 years[25] between 1976-1980 and 1988-1994 had all been reversed (Figure 3). There were no statistically significant differences in HSV-2 seroprevalence in non-Hispanic blacks between the 3 surveys (Figure 3).

Between 1976-1980 and 1999-2004, HSV-1 seroprevalence has been decreasing in both non-Hispanic whites and non-Hispanic blacks. When all age groups were combined, the test for trend was significant for non-Hispanic whites ($P<.001$) and non-Hispanic blacks ($P<.001$; FIGURE 4); the gaps in HSV-1 seroprevalence be-

Table 3. Changes in Weighted Herpes Simplex Virus 2 Seroprevalence Between NHANES in 1988-1994 and 1999-2004 in Persons Aged 17 to 49 Years After Adjustment*

	Adjusted HSV-2 Seroprevalence, %					
	Males			Females		
	NHANES			NHANES		
	1988-1994 (n = 3597)	1999-2004 (n = 3478)	Change (95% CI)	1988-1994 (n = 3932)	1999-2004 (n = 3838)	Change (95% CI)
Overall	16.6	11.3	−31.9 (−44.4 to −19.4)†	28.3	22.8	−19.4 (−27.1 to −11.8)†
Age group, y						
17-19	6.0	1.0	−83.3 (−96.5 to −70.1)†	12.4	4.9	−60.5 (−81.9 to −39.1)†
20-29	12.8	6.1	−52.3 (−74.1 to −30.5)†	23.4	15.4	−34.2 (−48.0 to −20.4)†
30-39	22.0	13.2	−40.0 (−54.4 to −25.6)†	29.6	26.4	−10.8 (−23.8 to 2.2)
40-49	16.5	15.9	−3.6 (−31.2 to 23.9)	35.3	30.0	−15.0 (−27.5 to −2.6)‡
Race/ethnicity						
Non-Hispanic white	14.6	9.2	−37.0 (−52.5 to −21.5)†	23.4	18.6	−20.5 (−31.5 to −9.5)†
Non-Hispanic black	27.5	24.2	−12.0 (−29.2 to 5.2)	51.3	46.1	−10.1 (−19.5 to −0.8)‡
Mexican American	18.6	11.0	−40.9 (−57.6 to −24.2)†	32.5	18.9	−41.8 (−52.9 to −30.8)†

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.
*Adjusted for age, race/ethnicity, poverty level, number of lifetime partners, marital status, and education level.
†$P<.005$.
‡$P<.05$.

tween non-Hispanic whites and non-Hispanic blacks were about 18 percentage points in all 3 surveys.

COMMENT

The seroprevalence of HSV-2 in the United States decreased by 19% in persons aged 14 to 49 years over an average interval of 10.5 years. The change in HSV-2 seroprevalence contrasts sharply with data from a previous report, which found that HSV-2 seroprevalence increased 30% between the 2 NHANES conducted in 1976-1980 and 1988-1994.[25] Our study suggests that the trajectory of increasing HSV-2 seroprevalence in the United States has been reversed.

Because HSV-2 is a lifetime infection, any increases or decreases in HSV-2 seroprevalence are expected to be first seen in younger persons. In adolescents and younger adults, HSV-2 seroprevalence is a cumulative measure of re-

Table 4. Changes in Weighted Herpes Simplex Virus 1 Seroprevalence in Persons Aged 14 to 49 Years Between NHANES in 1988-1994 and 1999-2004

	NHANES				
	1988-1994		1999-2004		
	Sample Size	HSV-1 Seroprevalence, % (95% CI)	Sample Size	HSV-1 Seroprevalence, % (95% CI)	Change, % (95% CI)
Overall*	9167	62.0 (59.6 to 64.6)	11 508	57.7 (55.9 to 59.5)	−6.9 (−11.6 to −2.3)†
Age group, y					
14-19	1788	45.7 (42.0 to 49.7)	4650	39.0 (36.8 to 41.3)	−14.7 (−23.1 to −6.2)‡
20-29	2750	56.2 (52.5 to 60.2)	2412	54.4 (51.8 to 57.1)	−3.2 (−11.1 to 4.7)
30-39	2567	65.7 (62.0 to 69.7)	2251	63.5 (60.8 to 66.5)	−3.3 (−10.3 to 3.6)
40-49	2062	72.6 (69.0 to 76.5)	2195	65.3 (62.6 to 68.0)	−10.1 (−15.8 to −4.3)‡
Sex by age group, y					
Male					
All ages*	4423	59.0 (55.9 to 62.3)	5511	55.9 (53.8 to 58.2)	−5.3 (−11.4 to 0.9)
14-19	848	43.3 (38.3 to 48.9)	2368	36.8 (34.3 to 39.4)	−15.2 (−26.8 to −3.7)†
20-29	1332	50.8 (46.1 to 56.0)	1044	51.7 (48.6 to 55.1)	1.8 (−9.6 to 13.2)
30-39	1203	61.9 (56.8 to 67.6)	1005	63.5 (59.6 to 67.6)	2.6 (−8.1 to 13.2)
40-49	1040	72.3 (68.6 to 76.2)	1094	62.9 (58.8 to 67.3)	−13.0 (−20.2 to −5.8)‡
Female					
All ages*	4744	65.0 (61.9 to 68.3)	5997	59.5 (57.6 to 61.4)	−8.5 (−13.7 to −3.2)‡
14-19	940	48.1 (42.6 to 54.3)	2282	41.4 (38.3 to 44.7)	−13.9 (−26.0 to −1.9)†
20-29	1418	61.7 (57.3 to 66.4)	1368	57.1 (53.6 to 60.9)	−7.5 (−16.2 to 1.3)
30-39	1364	69.6 (65.6 to 73.8)	1246	63.7 (60.6 to 67.0)	−8.6 (−15.5 to −1.7)†
40-49	1022	73.0 (68.0 to 78.3)	1101	67.5 (64.6 to 70.6)	−7.5 (−15.0 to 0)
Race/ethnicity by age group, y*§					
Non-Hispanic white					
All ages*	2652	56.6 (53.8 to 59.5)	4311	50.1 (47.8 to 52.6)	−11.5 (−17.5 to −5.5)‡
14-19	461	38.1 (33.9 to 42.8)	1220	30.7 (27.6 to 34.1)	−19.7 (−32.0 to −7.4)†
20-29	675	49.4 (44.6 to 54.7)	1042	46.1 (42.2 to 50.3)	−6.7 (−18.7 to 5.4)
30-39	792	61.2 (56.6 to 66.3)	1070	56.4 (52.7 to 60.4)	−8.0 (−17.2 to 1.2)
40-49	724	68.8 (64.3 to 73.7)	979	58.5 (55.0 to 62.1)	−15.0 (−22.4 to −7.5)‡
Non-Hispanic black					
All ages*	3009	74.0 (71.3 to 76.8)	2926	68.3 (65.1 to 71.7)	−7.7 (−13.2 to −2.2)†
14-19	599	57.2 (52.3 to 62.6)	1470	55.7 (51.9 to 59.7)	−2.6 (−13.3 to 8.0)
20-29	891	70.8 (66.3 to 75.7)	476	55.5 (49.9 to 61.8)	−21.6 (−31.1 to −12.1)‡
30-39	884	76.4 (72.5 to 80.5)	460	75.2 (70.4 to 80.3)	−1.6 (−9.5 to 6.4)
40-49	635	84.0 (80.8 to 87.4)	520	79.8 (75.0 to 85.0)	−5.0 (−11.7 to 1.7)
Mexican American					
All ages*	3113	86.0 (83.6 to 88.4)	3406	80.8 (78.7 to 83.0)	−6.0 (−9.6 to −2.5)‡
14-19	636	71.4 (66.2 to 76.9)	1658	57.6 (54.8 to 60.5)	−19.5 (−26.4 to −12.6)‡
20-29	1072	84.1 (81.7 to 86.6)	678	80.0 (75.4 to 84.9)	−4.9 (−10.9 to 1.1)
30-39	793	89.4 (86.7 to 92.0)	505	86.3 (82.6 to 90.3)	−3.5 (−8.4 to 1.4)
40-49	612	92.5 (88.6 to 96.6)	565	89.2 (86.5 to 92.1)	−3.6 (−8.4 to 1.3)

Abbreviations: CI, confidence interval; HSV-1, herpes simplex virus type 1; NHANES, National Health and Nutrition Examination Survey.
*Age-adjusted using the 2000 US census civilian, noninstitutionalized population aged 14 to 49 years as the standard.
†$P<.05$.
‡$P<.005$.
§For the 1988-1994 survey, 393 persons in the "other" race/ethnicity category were excluded; for the 1999-2004 survey, 865 persons in the "other" race/ethnicity category were excluded.

cent exposures. The decrease in HSV-2 seroprevalence in adolescents and young adults provides biological evidence supporting findings from behavioral surveys that sexual risk behaviors decreased in adolescents.[36] Seroprevalence of HSV-2 is not subject to self-reporting biases, and is a more direct measure of risk for sexually acquired HIV infection. Because audio computer-assisted self-interviewing increases the reporting of sexual behaviors in adolescents,[37] the change in methods likely made the reporting of sexual activities more complete in 1999-2004. As a result, the observed decreases in sexual behaviors in adolescents between the 2 surveys are likely to be underestimates.

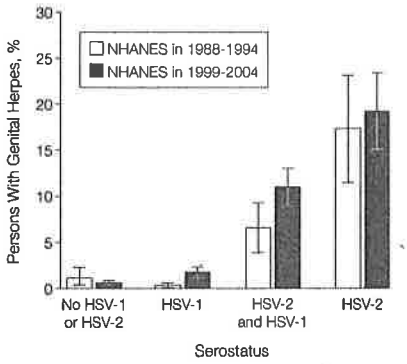
The reasons that HSV-2 seroprevalence significantly decreased even after accounting for changes in measured sexual behaviors may include a combination of unmeasured factors, such as careful partner selection, condom use, and/or choosing oral sex over vaginal sex[36,38-41]; additionally, adjusting for individual-level behavioral changes may not fully adjust for their population effects. Recent research into the structure of sexual networks indicates that in adolescents, relative low levels of behavioral change can radically limit the spread of sexually transmitted infections.[42] The finding that the mean number of lifetime sex partners increased in adult females but did not change in adult males may have contributed to the greater decreases in HSV-2 seroprevalence in males relative to females between the 2 surveys.

The overall seroprevalence of HSV-1 decreased by 7% between 1988-1994 and 1999-2004. During the same interval, there was an increase in persons infected with HSV-1 only who had been diagnosed with genital herpes. Our findings are consistent with previous reports that genital herpes caused by HSV-1 may be increasing in the United States, as in other developed countries.[6-13]

A decrease in HSV-1 seroprevalence in the United States is not unexpected due to improvements in living and hygiene conditions and experiences in other developed countries. In European countries, HSV-1 seroprevalence was inversely correlated with the national gross domestic product.[43] In the United Kingdom and in the Netherlands, the seroprevalence of HSV-1 has decreased since the late 1980s.[44,45] In Japan, changes in socioeconomic status and family size were associated with decreases in HSV-1 seroprevalence.[46] The overall seroprevalence of HSV-1 was unchanged between 1976-1980 and 1988-1994, probably because immigrants from countries with high HSV-1 seroprevalence offset the decrease in HSV-1 seroprevalence in those born in the United States.[26] Our finding that HSV-1 seroprevalence decreased overall by 7% but by 10% in those born in the United States supports this hypothesis.

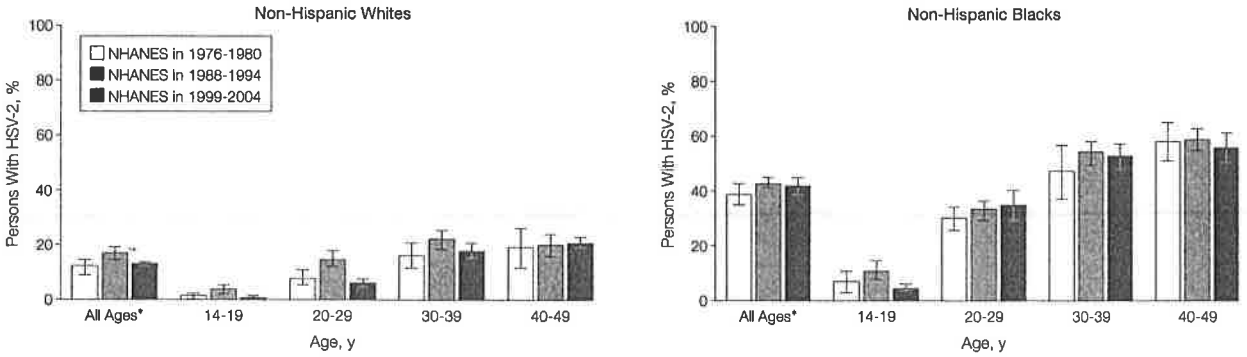
The changing HSV-1 seroprevalence may have an impact on genital herpes for several reasons. First, declines in HSV-1 acquisition before onset of sexual activity will leave more individuals susceptible to genitally acquired HSV-1 infection when they become sexually active. Second, more individuals would acquire HSV-2 infection with-

Figure 2. Persons Aged 18 to 49 Years Who Had Been Diagnosed With Genital Herpes, by Herpes Simplex Virus Serostatus on NHANES in 1988-1994 and 1999-2004



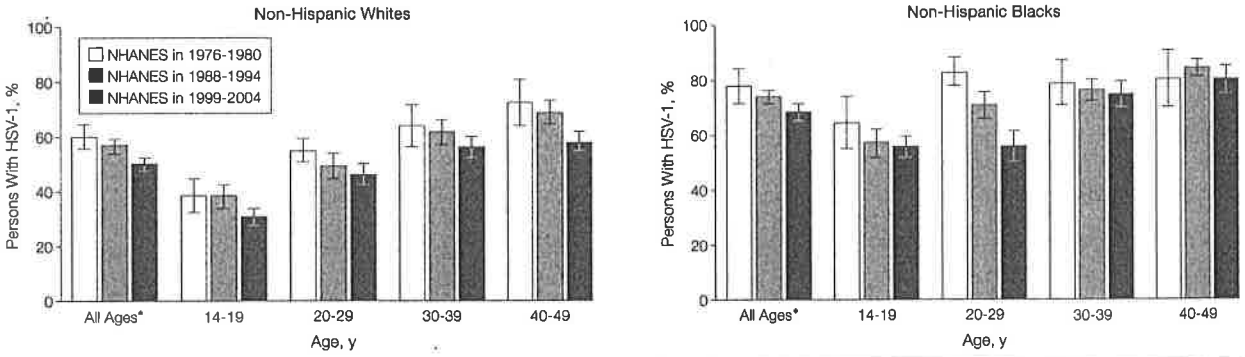
Error bars indicate 95% confidence intervals; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.

Figure 3. Herpes Simplex Virus Type 2 Seroprevalence in Non-Hispanic Whites and Non-Hispanic Blacks by Age, on NHANES in 1976-1980, 1988-1994, and 1999-2004



The percentage of persons is weighted. Error bars indicate 95% confidence intervals; HSV-2, herpes simplex virus type 2; NHANES, National Health and Nutrition Examination Survey.
*Age-adjusted using the 2000 US Census civilian, noninstitutionalized population aged 14 to 49 years as the standard.

Figure 4. Herpes Simplex Virus Type 1 Seroprevalence in Non-Hispanic Whites and Non-Hispanic Blacks by Age, on NHANES in 1976-1980, 1988-1994, and 1999-2004



The percentage of persons is weighted. Error bars indicate 95% confidence intervals; HSV-1, herpes simplex virus type 1; NHANES, National Health and Nutrition Examination Survey.
*Age-adjusted using the 2000 US Census civilian, noninstitutionalized population aged 14 to 49 years as the standard.

out HSV-1 antibodies. Despite the overall decrease in HSV-2 seroprevalence between 1988-1994 and 1999-2004, the seroprevalence of HSV-2 only (the group of persons with the highest medical burden of genital herpes) did not change. Finally, because genital HSV-1 recurs relatively infrequently, confirmation of HSV type may contribute to optimal management of patients with symptomatic genital herpes.[47,48]

One limitation of our study is the small number of questions about sexual behaviors in NHANES. We cannot compare rates of condom use because such data were not collected in the surveys. Another limitation is that the interpretation of national trends can be problematic for Mexican Americans due to the recent arrival of a large number of immigrants in the United States.[49]

While our data show declines in HSV-2 seroprevalence, HSV-2 is still prevalent in the United States. Furthermore, the gaps in HSV-2 seroprevalence between the sexes are widening and the disparity between racial groups persists despite the recent decrease in HSV-2 seroprevalence. There are several additions to the armamentarium for control of HSV-2 infection. A candidate vaccine that is partially protective in women has been reported.[24] Once-daily HSV-2 suppressive therapy can cut the risk of HSV-2 transmission in discordant couples by half,[50] and condoms use can also reduce the risk of transmission.[39,40]

Type-specific serological tests for HSV have become available[51] and can be used to identify populations in need of intensified interventions. Trials are ongoing to assess the impact of HSV-2 suppressive therapy on HIV acquisition.[52] If effective, HSV-2 suppressive therapy may become a new biomedical intervention limiting the spread of HIV as well as HSV-2 infection. The percentage of HSV-2 seropositive persons who had been diagnosed with genital herpes was higher in 1999-2004 compared with 1988-1994. However, the vast majority of persons seropositive for HSV-2 did not know that they were infected. These data are consistent with the lack of widespread testing in the general population.

NHANES provide a measure of population burdens of both HSV-1 and HSV-2 infections. In populations in which childhood acquisition of HSV-1 is common, such as in non-Hispanic blacks and Mexican Americans, sexual transmission of HSV-1 would be minimal. However, in populations whose acquisition of HSV-1 during childhood is relatively low and who have relatively low HSV-2 seroprevalence, such as non-Hispanic whites, sexual transmission of HSV-1 could become an increasingly important cause of genital herpes in the future. Recent changes in adolescent sexual behavior (particularly the practice of oral-genital sex) and the possible evolution of HSV-1 may accelerate this shift.[41,42,53] The ability to monitor HSV-1 seroprevalence along with HSV-2 seroprevalence in NHANES is important for the development of HSV-2 prevention strategies, such as those related to vaccination.[24] The changes in HSV-1 and HSV-2 seroprevalence will also directly affect the cause of neonatal herpes.[54] Future studies are needed to monitor the changing HSV-1 and HSV-2 trends and to develop effective strategies to prevent HSV infection.

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Study concept and design: Xu, Kottiri, McQuillan, Nahmias, Markowitz.
Acquisition of data: Xu, Kottiri, McQuillan, Lee.
Analysis and interpretation of data: Xu, Sternberg, Kottiri, McQuillan, Lee, Nahmias, Berman, Markowitz.
Drafting of the manuscript: Xu, Sternberg, Kottiri, Nahmias.
Critical revision of the manuscript for important intellectual content: Xu, Sternberg, Kottiri, McQuillan, Lee, Nahmias, Berman, Markowitz.
Statistical analysis: Xu, Sternberg, Kottiri, McQuillan.
Administrative, technical, or material support: Kottiri, McQuillan, Lee, Berman.
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rus 1 and herpes simplex virus 2 serology collected through the National Health and Nutrition Examination Surveys (NHANES). After the data became available, the Epidemiology and Surveillance Branch analyzed the data and developed the manuscript. The manuscript was reviewed and approved by the Division of STD Prevention, the Centers for Disease Control and Prevention, and then cross-clearance and approval was granted by the National Center for Health Statistics, Centers for Disease Control and Prevention.

Disclaimer: The findings and conclusions reported in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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FARLEY

EXHIBIT C

Seroprevalence of herpes simplex virus type 1 (HSV-1) in New York City, by sex and age group
Source: New York City Health and Nutrition Examination Survey (NYC HANES), 2004
Data are weighted to the 2000 New York City population.
Prepared by: Bureau of Epidemiology Services, New York City Department of Health and Mental Hygiene, July 2012

	Overall			Males			Females		
	N	%	95% CI	N	%	95% CI	N	%	95% CI
Total[1]	4,233,000	73.1	69.8-76.1	1,877,000	70.4	66.1-74.3	2,356,000	75.4	71.4-79.0
Age group[2]									
20-29	656,000	56.9	50.5-63.0	272,000	49.9	41.9-57.9	384,000	63.1	55.5-70.0
30-39	948,000	71.8	66.2-76.7	424,000	67.5	58.4-75.5	525,000	75.6	69.0-81.2
40-49	892,000	75.2	69.6-80.1	381,000	75.0	66.7-81.8	511,000	75.4	68.1-81.5
50-59	687,000	75.6	68.5-81.5	270,000	72.8	60.6-82.3	416,000	77.5	69.4-84.0
60-69	487,000	80.0	71.0-86.7	245,000	82.8	69.7-91.0	242,000	77.3	64.3-86.6
>70	563,000	86.8	77.9-92.4	286,000	83.8*	68.5-92.5	278,000	90.0*	77.7-95.9
Childbearing age (20-44) years	--	--	--	--	--	--	1,217,000	72.7	68.0-76.8

Notes:

[1]Among adults aged 20+ years. Age-adjusted to the US 2000 Standard Population.

[2]Age specific estimates are NOT age-adjusted.

NYC HANES data are comprised of 3 overlapping samples (Clinic/Home, Clinic, and Fasting), each of which is weighted to represent the total population of NYC adults aged 20+ years (N = 5, 827,719). Age distributions within each of the 3 samples vary slightly, producing unequal counts of the subpopulations of adults aged 20-75 years. All estimates are adjusted to account for component and item non-response.

*Estimate should be interpreted with caution. Estimate's Relative Standard Error (a measure of estimate imprecision) is greater than 30% or the sample size is too small, making the estimate potentially unreliable.

Confidence Intervals (CIs) are a measure of estimate precision: the wider the CI, the more imprecise the estimate.

Citation:

New York City Department of Health and Mental Hygiene. New York City Health and Nutrition Examination Survey [2004]: custom data table requested July 27, 2012.

Seroprevalence of herpes simplex virus type 1 (HSV-1) ONLY in New York City, by sex and age group[1]
Source: New York City Health and Nutrition Examination Survey (NYC HANES), 2004
Data are weighted to the 2000 New York City population. Age-specific estimates are NOT age-adjusted.
Prepared by: Bureau of Epidemiology Services, New York City Department of Health and Mental Hygiene, July 2012

	Overall			Males			Females		
	N	%	95% CI	N	%	95% CI	N	%	95% CI
Total[2]	3,007,000	51.6	48.6-54.6	1,506,000	56.0	51.9-60.0	1,501,000	47.8	44.0-51.6
Age group[3]									
20-29	577,000	50.0	44.3-55.7	259,000	47.5	39.7-55.3	318,000	52.2	45.4-59.0
30-39	735,000	55.6	50.1-61.0	369,000	58.9	49.8-67.4	365,000	52.6	45.7-59.4
40-49	587,000	49.5	44.3-54.7	294,000	58.0	49.8-65.7	293,000	43.2	36.5-50.0
50-59	413,000	45.4	37.8-53.3	182,000	49.1	38.7-59.5	230,000	42.9	33.9-52.3
60-69	284,000	46.6	38.1-55.4	168,000	56.8	43.5-69.1	116,000	37.0	26.5-48.9
>70	412,000	63.5	53.3-72.6	233,000	68.4*	52.9-80.7	179,000	58.0*	42.7-72.0
Childbearing age (20-44) years	--	--	--	--	--	--	867,000	51.7	47.3-56.2

Notes:

[1]Excludes those who were seropositive for HSV-2, HSV-1 and HSV=2, or were seronegative.

[2]Among adults aged 20+ years. Age-adjusted to the US 2000 Standard Population.

[3]Age specific estimates are not age-adjusted.

NYC HANES data are comprised of 3 overlapping samples (Clinic/Home, Clinic, and Fasting), each of which is weighted to represent the total population of NYC adults aged 20+ years (N = 5, 827,719). Age distributions within each of the 3 samples vary slightly, producing unequal counts of the subpopulations of adults aged 20-75 years. All estimates are adjusted to account for component and item non-response.

*Estimate should be interpreted with caution. Estimate's Relative Standard Error (a measure of estimate imprecision) is greater than 30% or the sample size is too small, making the estimate potentially unreliable.

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Citation:

New York City Department of Health and Mental Hygiene. New York City Health and Nutrition Examination Survey [2004]: custom data table requested July 27, 2012.